Technical paper

Optimization of the Production of Microparticulated Egg White Proteins as Fat Mimetic in Salad Dressings Using Uniform Design

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The aim of the current study was to reduce the fat content of salad dressings using microparticulated egg white proteins (MEWP) as fat mimetic. In this regard, the effects of heating and shearing conditions on textural properties, rheological properties, and color parameters of MEWP were investigated. Statistical analysis with uniform design methodology showed that there was a significant correlation between investigated responses and process variables ($P \leq 0.05$) except for rotational speed. Based on the optimal conditions (13 min for heat time, 3.6 for solution pH value, 90 g/L for protein addition amount, 60 s for shear time, 10000 r/min for rotational speed) produced by the optimization of process conditions, the textural and sensory properties, rheological properties, and color of MEWP were comparable to the commercial salad dressing (CSD). The results indicated that MEWP can be used as fat mimetic for the production of low-fat salad dressings.

Keywords: egg white protein, fat mimetic, salad dressing, uniform design

Introduction

Salad dressing is a kind of semi-solid oil-in-water emulsion product with a wide-spread consumption in worldwide (Nikzade *et al.*, 2012). It is made by mixing egg yolk, vinegar, oil, as well as spices, and usually contains 30 % to 80 % of fat, depending on different types of salad dressings. It has been widely recognized that over intake of fat will cause a number of diseases, for instance, obesity, diabetes, and breast cancer (Wu *et al.*, 2013). Fat mimetic, such as protein- and polysaccharide-based fat mimetic, can reduce the oil and cholesterol content in salad dressing (Diftis *et al.*, 2005; Yazici and Akgun, 2004).

The microparticulated egg white protein (MEWP), utilized as fat mimetic in salad dressings, was processed by heat treatment and microparticulation (Van der Plancken *et al.*, 2007). Since it involves many processing parameters, mainly

including egg white protein (EWP) addition amount, heat temperature, heat time, solution pH value, rotational speed and shear time, during the production of MEWP, the process optimization is difficult and key factors for improving the quality of MEWP is hard to be identified. A large amount of experiments are required to be conducted when applying the traditional optimization experimental design, such as orthogonal experiment and response surface methodology.

The experimental technique of uniform design is an excellent method firstly proposed by Mathematical Statistics expert Fang Kai-tai and Wang Yuan (2003). It is an experimental design method based on multivariate statistical and number theory (Luo, 2012) and allows the amount of levels for each factor as many as the number of experiment runs. Compared with the orthogonal experiment and response surface design, uniform design possess a better uniformity and

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less test number in the arrangement of multifactorial and multilevel experiments (Liang *et al.*, 2001). Therefore, it is less laborious and time-consuming than other approaches required to optimize a process. This experimental technique has been widely applied and achieved great benefits in the fields of military, medicine, textile, chemical, microbiological (Fang and Lin, 2003; Wang *et al.*, 2007). However, usage of uniform design in food processing, especially in the process optimization of formula products, is uncommon, thus it is very useful and promising to promote and popularize the application of uniform design in process and formula optimization of foods.

In the present work, uniform design was utilized to optimize the production of MEWP as fat mimetic in salad dressings, in order to obtain the MEWP fat mimetic closest to the commercial salad dressing (CSD) in terms of textural properties. The math models of relationships between textural properties (firmness, consistency, cohesiveness and the index of viscosity) and process parameters (heat temperature, heat time, solution pH value, rotational speed and shear time) were established, and the optimal conditions to produce MEWP were confirmed by the verified experiment. The comparison of MEWP and CSD were carried out by sensory evaluation, rheological and color measurements. Physical properties, including particle size and morphology, were conducted to further indicate the adaptability of MEWP used as fat mimetics in salad dressings.

Materials and Methods

Materials EWP, the regular egg white powder containing 84.45 % protein, was purchased from Kyushu Tin Shui Technology Co. Ltd (Beijing, China). Egg yolk (EY) powder was obtained from Tong He Biological Products Co. Ltd (He Bei, China). Food-grade glacial acetic acid was purchased from Yang Dong Chemical Industrial Company (Guang Dong, China), and citric acid was obtained from Zheng Zhou Taber Trading Co. Ltd (He Nan, China). McCormick salad dressing, a popular CSD containing 0.6 % protein, 34.9 % fat, and 27.0 % carbohydrate, was easily available from local market, thus it was chosen as a reference. The main ingredients of McCormick salad dressing included soybean oil, water, sugar, vinegar preparation, EY liquids, and other food additives. All other chemicals of analytical grade were purchased locally.

Production of the microparticulated egg white protein as fat mimetic The production of MEWP as fat mimetic in salad dressings was investigated by single-factor experiment and uniform design. Details of the production process are specified below:

At basic conditions EWP (90 g) and EY (0.5 g) powders were dispersed by deionized water (1 L). The pH value of EWP dispersion was adjusted by acetic acid and citric acid to 3.6, and then heated at 75 °C for 13 min without shaking. After heat treatment, the protein denatured and formed EWP gel (EWPG).

The protein gel was further stored at 0–5 °C for more than 12 h before microparticulation. The microparticulated egg white protein was obtained by shearing at a rotational speed of 10000 r/min for 60 s with a high-shear homogenizer (IKA T25, Germany) equipped with an IKA 0593400 rotor-stator generator. The rotor acts as a centrifugal pump to recirculate the liquid and suspended solid particles through the generator. The MEWP system was rapidly homogenized by shear, impact, collision, and cavitation (Hsieh *et al.*, 2014). The MEWP samples were stored at –18 °C for about 24 h before further instrumental and sensory analysis.

Experimental design In single factor experiments, the effect of changing levels of a factor on the textural properties of MEWP was studied with the other factors being held constant. Uniform design was then applied to determine the optimal process parameters of MEWP production. The investigated levels and factors were selected according to the results of single factor experiments. The combination effects of independent variables X_1 (heat time, min), X_2 (pH value), X_3 (protein addition, g/L), X_4 (shear time, s) and X_5 (rotational speed, r/min) at three changing levels and the results of dependent variables Y_1 (firmness, g), Y_2 (consistency), Y_3 (cohesiveness, g) and Y_4 (index of viscosity) of each experiment are shown in Table 1.

Textural profile analysis (TPA) The TPA test was determined using a TA-XT2 texture analyzer (Stable Micro Systems, England) equipped with a cylindrical probe with 35 mm diameter of disc plunger (A/BE35 probe). The Samples (50 mL) were placed into cylindrical containers (50 mm diameter) and axially compressed to 80 % of their original height with pre-test, test, and post-test speed of 1 mm/s, 1 mm/s, and 10 mm/s, respectively (Worrasinchai *et al.*, 2006; Yilmaz *et al.*, 2012). The disc plunger performs a compression test which extrudes the product up and around the edge of the disc. At the point of the maximum force, the plunger returned to its original position. The calculation of TPA values was obtained by graphing a curve using force and time plots. The parameters measured consisted of firmness (the maximum positive force of extrusion, g), consistency (area of the curve, g·s), cohesiveness (maximum negative force due to back extrusion, g), index of viscosity (negative area of extrusion, g·s), obtained by using the Texture Expert for Windows software version 1.20 (Stable Micro Systems). These textural properties were documented as indication of viscosity or consistency of samples (Kamboj and Rana, 2014).

Sensory evaluation Sensory evaluations of MEWP sample and CSD were conducted by 10 panelists, consisting of College of Food Engineering and Biotechnology staff and students, using a 10-point hedonic scale for two attributes (appearance, texture and flavor) where 10 is like extremely and 1 dislike extremely. The appearance was evaluated in terms of smoothness, gloss, and color; the texture indicator was described by the aspects of firmness, coarseness, and

Table 1. The scheme and results of uniform design experiments.

	X_1	X_2	X_3	X_4	X_5	Y_1	Y_2	Y_3	Y_4
1	12	3.5	11	90	10,000	266.14±2.49	9851.40±24.20	351.03±2.27	1136.55±29.53
2	14	3.3	10	120	10,000	254.57±5.84	9501.88±25.29	333.57±4.16	1089.55±15.83
3	16	3.5	9	60	10,000	230.18±2.54	8565.55±11.46	292.85±0.11	950.10±3.07
4	12	3.3	9	90	10,000	176.38±2.60	6421.83±26.21	224.29±3.03	723.63±24.62
5	14	3.7	11	120	11,000	186.38±2.38	6590.48±24.81	233.68±2.27	730.32±28.59
6	16	3.3	10	60	11,000	339.08±4.27	12495.56±64.76	431.38±1.41	1475.83±30.65
7	12	3.7	10	120	11,000	102.30±2.16	3370.36±61.23	122.09±1.95	406.26±6.94
8	14	3.3	9	60	11,000	277.87±5.46	10281.14±69.56	346.54±3.73	1145.16±0.15
9	16	3.7	11	90	12,000	292.42±5.24	10011.01±42.64	369.91±3.41	1112.91±58.59
10	12	3.5	11	120	12,000	206.88±4.00	7571.05±24.65	272.41±1.51	867.19±50.20
11	14	3.7	10	60	12,000	205.74±2.65	7742.77±13.17	253.27±3.35	839.43±2.92
12	16	3.5	9	90	12,000	202.12±1.30	7272.88±15.08	261.38±2.92	838.27±22.11

X_1 : heat time (min), X_2 : pH value, X_3 : protein addition (g/mL), X_4 : shear time (s), X_5 : rotational speed (r/min).

Y_1 : firmness (g), Y_2 : consistency (g·s), Y_3 : cohesiveness (g), Y_4 : index of viscosity (g·s).

consistency; the flavor was mainly assessed according to egg smell. The overall performance was calculated as a sum of the three indicators and water was provided for rinsing between samples.

Rheology Rheological properties of MEWP sample and CSD were taken using a HAAKE MARS III rheometer (Thermo-Scientific, Germany). The rheology measurements were carried out according to Liu *et al.*'s study (Liu *et al.*, 2007). A dependence of shear stress on shear rate was observed, and the shear rate was linearly increased from 0.1 to 150 s⁻¹. The experimental data were fitted by Herschel-Bulkley flow equation (Eq. 1).

$$\tau = \tau_0 + K\dot{\gamma}^n \quad \text{.....Eq. 1}$$

where τ is the shear stress (Pa); τ_0 is the yield stress (Pa); K is the consistency coefficient (Pa sⁿ); $\dot{\gamma}$ is shear rate (s⁻¹); n is the flow behavior index (dimensionless). Newtonian yield stress= $\tau_0=0$, $n=1$, pseudo-plastic yield stress= $\tau_0=0$, $n < 1$, dilatant yield stress= $\tau_0=0$, $n > 1$, and Bingham yield stress= τ_0 =value (Thaibudom and Khantarat, 2011). The data of rheological measurements were analyzed using the Rheowin Data Manager Version 4.30.

Color determination CIE-LAB tristimulus color values, including lightness (L^*), redness (a^*), and yellowness (b^*) of MEWP samples and CSD were evaluated by using a colorimeter (WSC-S, Shanghai Sheng Ke instrument equipment Co., Ltd., China) The color changes were quantified by the tristimulus color values (L^* , a^* , b^*) (McGuire, 1992).

Particle size distribution Particle size distributions of EWP, EWPG, and MEWP samples were determined using laser diffraction method on a BT-9300S Laser Particle Size Analyzer (Bettersize Instruments Ltd., Dandong, China).

Scanning electronic microscopy Structures of EWP, EWPG, and MEWP samples were analyzed by scanning

electron microscopy (SU-1510, Hitachi, Pleasanton, CA, USA). Samples were mounted on SEM aluminum stubs using double sided adhesive tape to which the samples were fixed and coated with a thin layer of gold using a sputter-coater (Blazer, SCD004). Magnification $\times 3000$ was used during micrograph analysis.

Statistical analysis Tests were performed in triplicate. Figures were illustrated as one of the parallel experiments and data were presented as the mean $\pm$ SD. Results were evaluated by one-way ANOVA, followed by Student's test for statistical analysis. Differences were considered statistically significant at $P < 0.05$. The variance analyses of uniform design results were based on the F statistic by using Statistical Package for Social Sciences (SPSS) v.14 software.

Results and Discussion

Single-factor experimental results The TPA test was performed for investigating the consistency of MEWP samples and CSD because consistency is commonly the textural property possessed by viscous products, such as yoghurt, creams, and sauces. Furthermore, consistency relates to the 'firmness', 'thickness' or 'viscosity' sensory of liquid or fluid semi-solid. These textural properties, including consistency, firmness, cohesiveness, and index of viscosity, represent the physical properties of fat mimetic, and are highly correlated with the sensory of dairy products (Liu *et al.*, 2007; Sandoval-Castilla *et al.*, 2004). Thus, textural properties were the target to optimize MEWP as fat mimetic closest to CSD.

The effect of heat time on the textural properties of MEWP samples is shown in Fig. 1A. This experiment adopted 10, 12, 14, 16, 18 and 20 min of heat treatment, and other experimental conditions were as follows: solution pH value, 3.5; EWP addition amount, 100 g/L; shear time, 90 s; rotational speed, 11,000 r/min. The result showed that all the textural properties,

Fig. 1. Effects of heat time (A), solution pH value (B), EWP addition amount (C), shear time (D) and rotational speed (E) on the textural properties of MEWP samples. Textural properties contain firmness (—▲—), cohesiveness (—△—), consistency (—■—) and index of viscosity (—□—).

including firmness, consistency, cohesiveness, and index of viscosity, increased with heat time. There is no difference on the textural properties of MEWP samples for heating of 14 min and 16 min, and a decrease occurred when heating for 20 min. This might be due to that protein denaturation led to the formation of coarse coagulum with low viscoelastic properties (Croguennec *et al.*, 2002).

The effect of solution pH value on the textural properties of MEWP samples is shown in Fig. 1B. As reported in previous studies, near the pI value of most EWP (in the range of 4.0–6.0), protein denaturation led to a coarse aggregation and the coagulum have low viscoelastic properties and low water-holding capacity, whereas EWP gels formed at pH 7 and 9 are more viscoelastic (Croguennec *et al.*, 2002). Due to partially hydration of protein at low pH levels, the thermal crosslinking of protein molecules was weakened, resulting in the weak gel

structure in samples (Holt *et al.*, 1984). However, taking the acidity taste of common salad dressings into account, this experiment adopted pH 3.1, 3.3, 3.5, 3.7, 3.9, and 4.1, while other experimental conditions were as follows: heat time, 16 min; EWP addition amount, 100 g/L; shear time, 90 s; rotational speed, 11000 r/min. The result showed that all the textural properties, including firmness, consistency, cohesiveness, and index of viscosity, gradually reduced with the increase of solution pH value, and the discrepancy between experimental results at difference pH values is significant.

The effect of EWP addition on the textural properties of MEWP samples had been investigated when different addition amounts of EWP were set as 80, 90, 100, 110, 120, and 130 g/L under the other experimental conditions as follows: heat time, 16 min; solution pH value, 3.5; shear time, 90 s; rotational speed, 11000 r/min. As shown in Fig. 1C, the

firmness, consistency, cohesiveness, and index of viscosity firstly increased along with increase of EWP addition amount from 80 g/L to 90 g/L, then kept at a stable level from 90 g/L to 110 g/L, and dramatically rose up after 110 g/L.

The effect of shear time on the textural properties of MEWP samples is shown in Fig. 1D. In this study, EWP were heated and then microparticulated in different shear time (30, 60, 90, 120, 150, and 180 s). Other experimental conditions were as follows: heat time, 16 min; solution pH value, 3.5; EWP addition, 100 g/L; rotational speed, 11000 r/min. The change trends of firmness, consistency, cohesiveness, and index of viscosity were identical. They decreased with the shear time from 30 s to 120 s and then increased after the shear time over 120 s.

The effect of rotational speed on the textural properties of MEWP samples had been studied in this work when different rotational speed (9,000, 10,000, 11,000, 12,000, 13,000 and 14,000 r/min) was set under the process conditions as follows: heat time, 16 min; solution pH value, 3.5; EWP addition amount, 100 g/L; and shear time, 90 s. As shown in Fig. 1E, the result implied the value of textural properties decreased with the rotational speed increasing from 9000 r/min to 12000 r/min, and then increased until the rotational speed reached to 13,000 r/min. But when the rotational speed was set as 13000 r/min, the values of textural properties began to go downward. The rotational speed is too fast, so that the resulting protein gels were broken into tiny particles and the stability of dispersion system was destroyed, leading to poor textural quality of products.

The textural properties of CSD were determined in a certain range as follows: firmness, 100.51–160.60 g; consistency, 4859.84–5481.64 g·s; cohesiveness, 114.06–200.65 g; index of viscosity, 400.62–609.03 g·s. The result suggested that MEWP samples with textural properties varying in the above range could be used as fat mimetic in salad dressings. Therefore, in the next uniform experiments, we adopted 12–16 min of heat time, 3.3–3.7 of solution pH value, 90–110 g/L of EWP addition, 60–120 s of shear time and 10000–12000 r/min of rotational speed for further uniform design.

Data analysis of Uniform Design The uniform design method was utilized for highly efficient optimization of the operation variables and levels in the production of MEWP. Based on the variance analysis, the insignificant independent variables were firstly eliminated, and then a regression analysis was carried out to fit the mathematical model to experimental data (Dai and Wang, 2015). Generally, multiple linear regression analysis and stepwise regression analysis are applied to uniform test analysis (Jiang and Ai, 2014). In this study, X_5 (rotational speed) was excluded as it showed no significance on each of the dependent variable. In addition, as each factor was highly correlated with each index on the basis of single factor experiments, multiple linear regression analysis was carried out

to establish the linear regression equation of independent variables (heat time, X_1 ; solution pH value, X_2 ; EWP addition amount, X_3 ; shear time, X_4) and textural properties (firmness, Y_1 ; consistency, Y_2 ; cohesiveness, Y_3 ; index of viscosity, Y_4). The predicted linear regression equations (Eq. 2–5) are given as follows:

$$Y_1 = 416.177 + 17.973X_1 - 274.70X_2 + 63.577X_3 - 1.264X_4 \quad \cdots \cdots \text{Eq. 2}$$

$$Y_2 = 18855.183 + 605.078X_1 - 10781.014X_2 + 2329.462X_3 - 50.90X_4 \quad \cdots \cdots \text{Eq. 3}$$

$$Y_3 = 575.650 + 23.359X_1 - 378.778X_2 + 85.064X_3 - 1.517X_4 \quad \cdots \cdots \text{Eq. 4}$$

$$Y_4 = 2327.896 + 70.706X_1 - 1317.973X_2 + 273.199X_3 - 5.488X_4 \quad \cdots \cdots \text{Eq. 5}$$

The regression equations are quaternary linear equations, and the variance analyses of the equations are shown in Table 2. The coefficients of determination (R^2) of the predicted equations were 0.959, 0.967, 0.957, and 0.973, respectively, suggesting a good fit, and the predicted equations could represent the resulted data of uniform design. Thus, the regression equation was successfully established to explain the changing relationship between textural properties and operation parameters. As shown in Table 2, the regression coefficient variance of four independent variables for different textural properties showed extremely significant with $P < 0.01$. In addition, the significance of each coefficient was determined in Table 2. The corresponding variables will be more significant if significance value becomes smaller by variance analysis (Wu and Xu, 2011). By comparing the significance values of different independent variables for each dependent variable, it can be seen that the impact of independent variables on firmness were EWP addition amount (X_3) > solution pH value (X_2) > heat time (X_1) > shear time (X_4); the impact of independent variables on consistency were solution pH value (X_2) > EWP addition amount (X_3) > shear time (X_4) > heat time (X_1); the impact of independent variables on cohesiveness were EWP addition amount (X_3) > solution pH value (X_2) > heat time (X_1) > shear time (X_4); the impact of independent variables on the index of viscosity were solution pH value (X_2) > EWP addition amount (X_3) > shear time (X_4) > heat time (X_1).

Then the optimal parameters for the production of MEWP as fat mimetic in salad dressings were determined by linear programming, aiming at the textural properties of MEWP closet to CSD. Due to the different textural values for different salad brands, McCormick salad dressing was selected as references for the linear programming analysis. An optimum process parameters were found as follows: heat time of 13 min, solution pH value of 3.6, EWP addition amount of 90 g/L, shear time of 60 s. Based on cost considerations, the lowest level of rotational speed (10,000 r/min) was chosen as it was not significant on each of the dependent variable.

To verify the successful math model, three random groups of test, excluding the tests in the uniform design scheme (Table 1), were carried out. The error of each experimental value

Table 2. The variance analysis of uniform design results.

	Sources	Sum of squared deviations	Degrees of freedom	Variance ^a	F ratio ^b	Significance
Y_1 (Firmness)	X_1	7.7103×10^3	2	3.8552×10^3	9.0604	0.0535
	X_2	1.7611×10^4	2	8.8055×10^3	20.6948	0.0176**
	X_3	1.9292×10^4	2	9.6460×10^3	22.6702	0.0155**
	X_4	7.1713×10^3	2	3.5857×10^3	8.4271	0.0587
	Error	1.2765×10^3	3	425.4938		
	Regression	4.1192×10^4	4	1.0298×10^4	41.0330	0.0001**
	Residual	1.7568×10^3	7	250.9669		
	Sum	4.2948×10^4	11			
Y_2 (Consistency)	X_1	9.2722×10^6	2	4.6361×10^6	11.2199	0.0405*
	X_2	2.7292×10^7	2	1.3646×10^7	33.0245	0.0091**
	X_3	2.5955×10^7	2	1.2978×10^7	31.4077	0.0097**
	X_4	1.1483×10^7	2	5.7415×10^6	13.8951	0.0304*
	Error	1.2396×10^6	3	4.1320×10^5		
	Regression	5.9013×10^7	4	1.4753×10^7	51.3329	0.0000**
	Residual	2.0118×10^6	7	2.8740×10^5		
	Sum	6.1025×10^6	11			
Y_3 (Cohesiveness)	X_1	1.3148×10^4	2	6.5739×10^3	9.6845	0.0491*
	X_2	3.3602×10^4	2	1.6801×10^4	24.7509	0.0137*
	X_3	3.4551×10^4	2	1.7275×10^4	25.4496	0.0131*
	X_4	1.0725×10^4	2	5.3627×10^3	7.9002	0.0637
	Error	2.0364×10^3	3	678.8071		
	Regression	7.0217×10^4	4	1.7554×10^4	39.2133	0.0001**
	Residual	3.1336×10^3	7	447.6571		
	Sum	7.3350×10^4	11			
Y_4 (Index of Viscosity)	X_1	1.2016×10^5	2	6.0081×10^4	11.4785	0.0393*
	X_2	4.0824×10^5	2	2.0412×10^5	38.9975	0.0071**
	X_3	3.6023×10^5	2	1.8012×10^5	34.4111	0.0085**
	X_4	1.3373×10^5	2	6.6863×10^4	12.7742	0.0341*
	Error	1.5703×10^4	3	5.2342×10^3		
	Regression	7.9917×10^5	4	1.9979×10^5	62.5667	0.0000**
	Residual	2.2353×10^4	7	3.1933×10^3		
	Sum	8.2152×10^5	11			

^aVariance was calculated by dividing a sum of squared deviations by its associated degrees of freedom. ^bF ratio was calculated by dividing the variance of independent variable by error variance, which is to test hypotheses about the effect of an independent variable on a dependent variable.

**indicates that the variance is significant at $P < 0.01$, * indicates that the variance is significant at $P < 0.05$.

relative to the predicted value was less than 5 %, indicating that the predicted model could represent observed values exactly and the regression models were constructed successfully. Furthermore, the textural and sensory properties of MEWP under the optimal operation parameters and CSD are shown in Table 3. The firmness, consistency, cohesiveness, and index of viscosity values of the optimal MEWP sample and CSD were 157.67 g and 159.87 g, 5997.29 g·s and 5339.05 g·s, 188.93 g and 189.79 g, 633.43 g·s and 612.06 g·s, respectively, which showed that MEWP had a similar textural property with that of

CSD. Considering the sensory evaluation, the appearance and texture of MEWP was comparable to that of CSD, but MEWP had slight egg smell due to the presence of egg yolk in samples without other additives addition, resulting in reduced sensory scores. Therefore, MEWP was an alternative choice as fat replacer for low-fat salad sauce products, having a similar appearance and texture to that of CSD and its overall performance was acceptable.

Comparison of the rheological properties of MEWP and CSD Rheological measurements were carried out for the

Table 3. Textural, sensory properties and Herschel-Bulkley model-fitted parameters of EWF and CSD.

Samples	Firmness (g)	Consistency (g·s)	Cohesiveness (g)	Index of Viscosity (g·s)	Appearance	Texture	Flavor	Overall performance	τ_0 (Pa)	K (Pa·s ⁿ)	<i>n</i>
MEWP	157.67±2.06	5997.29±28.35	188.93±2.38	633.43±20.70	8.0±0.4	7.8±0.5	7.7±0.5	23.5±1.4	0	93.5896	0.084
CSD	159.87±1.17	5339.05±11.43	189.79±4.71	612.06±3.61	8.3±0.6	8.2±0.7	9.0±0.3	25.5±1.6	0	56.1187	0.304

comparison of MEWP and CSD. Oscillatory test was conducted in a frequency range of 0.1–10 Hz within the linear viscoelastic region (1.0 Pa). The storage modulus (G'), loss modulus (G''), $\tan(\delta)$, and complex viscosity (η^*) were analyzed according to the results of oscillatory test (Fig. 2). As shown in Fig. 2A and B, G' values were significantly higher than G'' values, indicating that both MEWP and CSD exhibited a viscoelastic behavior like mayonnaise (Nikiforidis *et al.*, 2012). Fig. 2C showed that the $\tan(\delta)$ values of MEWP and CSD were less than 1.0, suggesting that MEWP and CSD behave as solid. However, an unstable fluctuation of $\tan(\delta)$ value occurred for MEWP and CSD at the frequency above 6.8 Hz. It might be attributed to the instable flow or edge rupture of samples between the measurement parallel plates, as parts of samples were thrown out at relatively higher frequency. In addition, the G' , G'' and $\tan(\delta)$ values of MEWP were relatively higher than those of CSD, which suggested MEWP showed more viscous behavior compared with CSD, as confirmed by the complex viscosity (η^*) results (Fig. 2D). The viscosity of MEWP was higher than that of CSD, and the η^* values for both MEWP and CSD were reduced with the frequency increasing.

Flow curves of MEWP and CSD are presented in Fig. 3A. A thixotropic loop was measured by first increasing the shear rate logarithmically from 0 to 150 s⁻¹, maintaining at 150 s⁻¹ for 240 s⁻¹, and finally decreasing logarithmically back to 0 s⁻¹. Fig. 3A showed that the value of downward curve was lower than that of upward curve at the same shear rate for MEWP and CSD, suggesting that both MEWP and CSD exhibited a thixotropic behavior. This result was in accordance with the previous report (Ma *et al.*, 2013), in which mayonnaise showed a thixotropic shear-thinning behavior. The area made up of shear rate and shear stress in thixotropic loop represents the reversibility of samples (Sikora *et al.*, 2015). The larger area means weaker thixotropy (Thaibudom and Khantarat, 2011). The thixotropic areas of MEWP and CSD were 5163.73 and 1321.57, respectively. The thixotropy of MEWP was weaker than CSD significantly, which indicated that it is more difficult for MEWP to return to the original state immediately when decreasing the shear rate.

In addition, to better understand the processing behavior, the rheological behavior was characterized by Herschel-Bulkley equation and the fitting results are summarized in Table 3. MEWP and CSD possessed the flow behavior index of 0.084 and 0.304, respectively, displaying non-Newtonian behavior with typically shear-thinning phenomenon. MEWP displayed relatively lower flow behavior index than that of CSD, and showed greater shear-thinning capacity. As the shear rate increased, emulsion droplets might aggregated to overcome hydrodynamic force and disrupted to cause a reduction of viscosity, consequently providing more resistance against flow and giving high shear-thinning capacity. Therefore, although the textural and sensory properties of MEWP are comparable

Fig. 2. Storage modulus G' (A), loss modulus G'' (B), $\tan(\delta)$ (C) value and η^* value (D) versus frequency for MEWP and CSD.

to those of the commercially available salad dressings, there remained a discrepancy between rheological properties of MEWP and CSD. In order to improve the thixotropy and rheological properties, xanthan gum (Dolz *et al.*, 2007), konjac glucomannan, and locust bean gum (Dolz *et al.*, 2007; Liu *et al.*, 2013) could be added as thickeners.

Comparison of the color of MEWP and CSD As is shown in Fig. 3B, the differences of brightness (L^* value) and redness (a^* value) between MEWP and CSD were insignificant ($P > 0.05$), whereas the yellowness (b^* value) of MEWP were significantly lower than that of commercial salad dressing ($P < 0.05$). In general, samples with high fat content and small fat droplet size have high L^* values, due to high refraction of light (Thaiudom and Khantarat, 2011), and the oil as well as egg yolk in CSD contribute a large amount of yellow color (Li *et al.*, 2014). In the further study, an appropriate amount of food colorants will be selected to improve MEWP color closest to those of CSD, especially for brightness and yellowness.

Physical properties of MEWP Physical properties, including particle size and morphology, of MEWP were performed to further indicate the adaptability of MEWP as fat mimetic in salad dressings. Particle size distributions of EWP samples are shown in Fig. 4A–C. The median particle diameters of EWP, EWPG and MEWP samples were 25.87, 33.87 and 9.42 μm , respectively. The increase of particle size

of EWPG from EWP was mainly because of the formation of huge and loose gel granules during heat treatment, and the size of EWPG increased with the growing of heating time and temperature (Nicorescu *et al.*, 2011). EWP sample formed into EWPG throughout denaturation and aggregation process, then formed into micro-sized MEWP particles by microparticulation treatment (Çakır-Fuller, 2015; Chung *et al.*, 2014). According to Ziegler (1992), the oral mucosa can detect the presence of protein particles of more than 5 μm in size. When the microparticulated proteins are small enough, the tongue cannot perceive them individually, but having the creamy texture of fat. As shown in Fig. 4B and 4C, the particle size of EWPG became smaller less than 10 μm after microparticulation, and the particle size distribution were more concentrated, bringing about a lubrication taste of MEWP as fat mimetic to simulate that of fat perfectly. In addition, protein particles with the diameter of more than 10 μm could also reassemble the texture of fats if the friction was reduced and the protein microparticles rolled and compressed with each other in the presence of a gum thickener, for example, xanthan (Liu *et al.*, 2018).

SEM was used to observe the change in size and morphology of EWP, EWPG and MEWP samples (Fig. 3D–F). As shown in Fig. 3D, the original EWP showed a uniform and globular particle distribution, as described by Tomczynska-Mleko (2015). It has found that some proteins were prone to

Fig. 3. Thixotropy loop flow curves (A), tristimulus color values (B) and photographs of MEWP and CSD in Petri dish (10 cm in diameter).

* indicates that the variance is significant at $P < 0.05$.

Fig. 4. Particle size distribution of EWP (A), EWPG (B) and MEWP (C); SEM images of EWP (D), EWPG (E) and MEWP (F).

form a fibrous gel when treated with heat, pH or cellulase hydrolysis (Tomczynska-Mleko *et al.*, 2015; Weijers *et al.*, 2006), or aggregated to form a network gel structure after thermal treatment (Lassé *et al.*, 2015; Vander Plancken *et al.*, 2006). In this study, the aggregated EWP formed a network structural EWPG under the optimal operation parameters, as shown in Fig. 4E. However, the gel structure surface is too rough and large to simulate the lubricating taste of CSD (Tabilo-Munizaga and Barbosa-Cánovas, 2005), thus microparticulation should be adopted to reduce the particle size of EWPG and make protein gel softer and smoother. According to Fig. 4F, MEWP was obtained by microparticulating EWPG network into tiny gel particles with the particle size less than 10 μm , which was in consistent with the result of particle size distribution.

Conclusions

Uniform design was effective experimental technique to understand the influence of operation parameters on textural properties of MEWP, and the resulting data are useful to optimize the production of MEWP as fat mimetic in salad dressings. The regression models are considered well fitted and explain the changing relationship between textural properties and operation parameters. The optimal operation parameters were as follows: heat time of 13 min, solution pH value of 3.6, EWP addition amount of 90/L, shear time of 60 s, rotational speed of 10,000 r/min. Under these conditions, the textural properties of MEWP produced are comparable to those of the commercially available salad dressings, and the overall performance was acceptable, but there remained a discrepancy between rheological properties of MEWP and CSD. The results of physical properties indicated that MEWP with particle size less than 10 μm provided a lubrication taste of fat mimetic. Therefore, MEWP could be an alternative choice as fat replacer in salad dressings.

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