Physicochemical, texture properties, and microstructure of yogurt using polymerized whey protein directly prepared from cheese whey as a thickening agent

Tianqi Fang¹ and Mingruo Guo^{2,3*}

¹Department of Food Science, College of Food Science and Engineering, Jilin University, Changchun, 130062, China

²Department of Nutrition and Food Sciences, College of Agriculture and Life Sciences, University of Vermont, Burlington 05405

³Department of Food Science, Northeast Agriculture University, Harbin, 150030, China

ABSTRACT

The aim of this study was to investigate suitability of polymerized whey protein prepared directly from cheese whey on the physicochemical, texture properties, and microstructure of the yogurt. The results indicated that addition of polymerized whey protein obtained by heating the liquid whey protein concentrate at 75°C for 10 min had no significant differences in pH, titratable acidity, total solids, protein content, viscosity, texture, and syneresis between the yogurt with polymerized liquid whey protein (YWPS) and the yogurt with polymerized whey protein concentrate. However, the YWPS had significant differences in viscosity, texture, and syneresis compared with the control yogurt. Scanning electron micrographs of YWPS displayed a compact and homogeneous protein network for polymerized whey protein solution (PWPS) samples. The 4 yogurt samples were evaluated by the quantitative descriptive analysis method, and 14 sensory attributes were analyzed by principal component analysis. All 3 principal components had significant effects on the sensory profiles, accounting for 52.3, 24.32, and 10.8% of the variability in the results, respectively. Polymerized whey protein prepared directly from cheese whey may be a good protein base as a thickening agent for yogurt making.

Key words: cheese whey, polymerized whey protein, thickening agent

INTRODUCTION

Cheese whey is a byproduct of cheese-making (Castro et al., 2009). Biochemical and chemical oxygen demand

of whey can cause environmental pollution (Yadav et al., 2015; Remón et al., 2016). Whey proteins, once thought to be the waste product of cheese making, have received a lot of attention from researchers not only because of their availability but also because they have certain desirable functional properties and high nutritional value and is often used to fortify protein in dairy products (Mulvihill and Ennis, 2003). The functional properties of whey protein can be improved by formation of polymerized whey protein (PWP), including emulsifying properties, foaming capacity, and thermal properties (Schmitt et al., 2007; Nicolai et al., 2011). Therefore, formation of PWP provides new opportunities to expand whey protein applications. Heat treatment has been used to improve emulsification properties of whey proteins including whey protein concentrate (WPC) and whey protein isolate (WPI) in the food industry (Mensi et al., 2013). In addition, the heat-induced aggregation of whey proteins may improve turbidity, viscosity, surface hydrophobicity, and gelation of whey proteins to achieve the desired structure and sensory properties of certain foods (Ren et al., 2017). It has been reported that PWP improved the body texture and enhanced the water-holding capacity of fermented dairy foods (Li and Guo, 2006).

Yogurt is one of the most consumed dairy products in the world (Cardines et al., 2018). Besides its nutritional value, the viscosity and rate of syneresis of yogurt are important indexes of sensory qualities and stability of yogurt products (Domagala et al., 2013). The most common means to improve the texture of yogurt is to increase the TS content of milk, which can be achieved by adding milk protein and solids (Zhang et al., 2015). Addition of PWP in yogurt making could improve the viscosity and syneresis of goat milk yogurt (Li and Guo, 2006). Native whey proteins are not commonly used as an ideal thickening agent because their solutions have lower viscosity because of the compact approximate globular structures and low molecular weight (Fitzsimons et al., 2008). However, heating can be used to

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*Corresponding author: mguo@uvm.edu

prepare soluble whey protein aggregates that enable whey protein to form high molecular weight complexes and thus increase the viscosity under certain conditions (Vardhanabhuti and Foegeding, 1999; Vardhanabhuti et al., 2001). Henriques et al. (2013) made it possible to incorporate liquid whey protein concentrates in set yogurt production, which was produced from cheese whey without expensive processing steps (e.g., evaporation and drying) for small- and medium-scale dairy plants. Therefore, we hypothesized that polymerized liquid whey protein concentrate prepared directly from Cheddar cheese whey may be a more effective thickening agent for full-fat yogurt production. By using this method, we may obtain an alternative way to prepare PWP through thermal aggregation of conventional dry products, such as WPC or WPI, in manufacturing fermented dairy products.

MATERIALS AND METHODS

Materials

High-temperature-sterilized cow milk ($\geq 8.2\%$ wt/vol nonfat solids, 5% wt/vol protein, and 6% wt/vol fat) was purchased from a local supermarket (Huishan Dairy Industry Co., Ltd., Changchun, China). Freeze-dried starter cultures containing *Streptococcus thermophilus* and *Streptococcus bifidobacterium* (ABY-3, thermophilic yogurt culture) was purchased from Chr. Hansen (Horsholm, Denmark). Whey protein concentrate (WPC80) was provided by Hilmar (Hilmar, CA). Liquid whey was collected from Cheddar cheese production. All other reagents were purchased from Beijing Chemical Works (Beijing, China).

Preparation of Liquid Whey Protein Concentrate Solution

The fresh whey from Cheddar cheese manufacture was pasteurized (60°C for 30 min) and prefiltered using screen mesh. The treated whey was subjected to microfiltration (0.1 μm). The microfiltered whey was ultrafiltered using a 10 kDa cut-off membrane to 25-fold. The UF-treated whey was electrodialyzed to remove 90% of salt, and the final protein content of the liquid whey protein concentrate solution (**WPS**) was 8.0% (wt/vol).

Preparation of PWP

The liquid whey protein solutions were adjusted to pH (7.0, 8.0, or 9.0) using NaOH (2 M). The solutions

were subjected to different treatment protocol, heated at different temperatures (70, 75, 80, 85, or 90°C) for 20 min at pH 7.0, heated at 75°C for different times (10, 20, 30, 40, 50, or 60 min) at pH 7.0, and heated at 75°C for 20 min at different pH values (7.0, 8.0, and 9.0). The obtained PWP solutions were kept at room temperature ($25 \pm 1^\circ\text{C}$) until further analysis. In this study, PWP (heated at 75°C for 20 min at neutral pH) was selected for yogurt making obtain a higher viscosity of yogurt based on preliminary experiment. The WPC powder (10 g) was suspended in 100 mL of Milli-Q water (Millipore Corp., Milford, MA) and kept at 4°C overnight. The WPC solution was adjusted to pH 8.0 using NaOH (2 M). The solution was heated at 85°C for 30 min as previously described (Shen et al., 2016).

Preparation of Yogurt Samples

Three batches of yogurt were produced with whole milk. Four different yogurt formulations were prepared: (1) conventional yogurt with no addition as a control, with (2) addition of polymerized whey protein solution (**PWPS**, directly prepared from cheese whey, 2%, vol/vol; **YWPS**), with (3) addition of WPS (liquid whey protein solution prepared from cheese whey, 2%, vol/vol; **UNYWPS**), and with (4) addition of polymerized whey protein prepared from commercial whey protein concentrate powder (**PWPC**, 2% vol/vol; **YWPC**).

The whole milk were preheated (60°C), mixed with sugars (7%, wt/vol), sterilized at 80°C for 20 min, cooled to 42°C, mixed with different whey protein solutions and yogurt starter (ABY-3), and fermented at $42 \pm 1^\circ\text{C}$ for 4 h. The yogurts were then stored at 4°C for 18 h after cooling. The production process of yogurt samples is shown as Figure 1.

Physicochemical Property Analysis

The TS, total protein, fat, ash, and titratable acidity were determined according to AOAC International (2002). The pH values of samples were measured using a pH meter (PHS-3C, Jingke, Shanghai, China). The TS content was measured using an infrared dryer (Moisture Analyzer MA30, Sartorius, Göttingen, Germany). Protein content was measured by the Kjeldahl method and fat was measured by the Gerber method. Ash content was measured by dry-ashing using a muffle furnace (SX-2.5-12; Jingke). The content of carbohydrate was determined by the difference of the TS content minus other solid component contents as previously described (Guzmangonzalez et al., 2000; Smith et al., 2016).

Viscosity and Texture Profile Analysis

After storage at 4°C for 18 h, yogurt samples were equilibrated at room temperature for 2 h. Viscosity was measured with a Brookfield viscometer (DV-III, Brookfield Engineering Labs Inc., Middleboro, MA) at 25°C. Texture analysis was conducted on a texture analyzer (Brookfield Engineering Labs Inc.) at 25°C. All measurements were performed using a fresh sample without breaking the original yogurt structure. A texture profile analysis model was run with a penetration distance of 30 mm using an acrylic cylindrical probe (diameter: 38.10 mm; height: 20 mm). The speed of the probe was

1 mm/s with a trigger of 4.5 g. The hardness, adhesiveness, springiness, and cohesiveness of samples were all recorded.

Syneresis Analysis

The syneresis of the yogurt samples was measured gravimetrically according to Lucey et al. (1998) with some modifications. The samples of yogurt (20 g) were transferred into 50-mL centrifugal tubes. Then the samples were centrifuged (Avanti J-E, Beckman Coulter, Brea, CA) at $1,200 \times g$ for 20 min (25°C), and the supernatants were removed and weighed. The syneresis

Figure 1. Schematic diagram of preparation process for different yogurt samples. Control = full-fat yogurt; YWPS = yogurt with addition of liquid polymerized whey protein (heated at 75°C for 20 min, pH 7.0); UNYWPS = yogurt with addition of unheated whey protein solids from cheese whey; YWPC = yogurt with heat-treated whey protein concentrate (heated at 80°C for 30 min, pH 7.0). PWPS = polymerized whey protein solution; WPS = liquid whey protein solution; PWPC = polymerized whey protein prepared from commercial whey protein concentrate (WPC 80). The ABY-3 was purchased from Chr. Hansen (Horsholm, Denmark).

Table 1. Definitions of the descriptors used in the sensory evaluation of the yogurt samples

Sensory descriptor	Definition	Reference sample ¹
Visual		
Color	Intensity of color ranging from yellow color to white	Erie Pure milk = 13
Brightness	Amount of light reflected from the product's surface	Erie Pure milk = 10, Nestlé condensed milk = 8
Syneresis	Presence of superficial liquid	Vigor curd milk = 12
Homogeneity	The absence of detectable particles that could be visually identified	Milkana triangle-shaped cheese = 14
Thickness	Observation of yogurt drop state	Diet fiber solid drink = 6
Odor		
Acid	Acid aroma characteristic of acid products	Vinegar = 13
Sweetness	Sweet aroma characteristic of sweet products	0.2% Vanillin = 5
Fermented milk	Typical aroma of milk that has been submitted to a fermentation process	Milkana triangle-shaped cheese = 11
Flavor		
Sweetness	Elemental taste produced by aqueous solutions of sugar and different sweeteners	80 g/L of sucrose solution = 8
Saltiness	Elemental taste produced by aqueous solutions of sodium chloride	1 g/L of sodium chloride solution = 4
Acidity	Elemental taste produced by aqueous solutions of citric acid substances	0.5 g/L of citric acid solution = 3.5, 1 g/L of citric acid solution = 7
Texture		
Decentrality	Degree of dispersion perceived when compressing the product between the tongue and the palate	Diet fiber solid drink = 12
Mouthcoating	The mouthfeel of the product, once swallowed, consists of the perception of a thin layer covering the palate.	Milkana triangle-shaped cheese = 8.5, diet fiber solid drink = 5
Smoothness	Geometric property related to the perception of the size and form of the particles	Milkana triangle-shaped cheese = 13

¹Erie Pure milk (Erie, Mongolia Yili Industrial Group Co. Ltd., Inner Mongolia, China), containing 3.2% protein, 3.8% fat, 5.0% carbohydrate, and higher than 8.5% of non-fat milk solids content, was purchased from local market. Nestlé condensed milk: Nestlé S.A., Vevey, Switzerland. Vigor curd milk: Dongguan Yiqun Food Co., Ltd., Dongguan, China. Milkana triangle-shaped processed cheese: BSI (Tianjin) Food Co., Ltd., Tianjin, China. Diet fiber solid drink: Jilin Senke Native Products Co., Ltd., Changchun, China.

was calculated as the percentage of the supernatant weight to yogurt weight.

Microstructure Analysis

Yogurt samples were examined by scanning electron microscopy according to the method previously described (Wang et al., 2017). Yogurt samples were fermented in the 2.5% agar cube (1 mm × 1 mm × 1 mm) taken from below the surface and were fixed in the cube, incubated at 43°C for 4 h, and then stored at 4°C overnight. The fixed samples were washed several times with 0.1 M phosphate buffer (pH 7.2). The samples were then dehydrated using a series of increasing ethanol concentrations (20, 40, 60, 70, and 90%) finishing with 3 changes of absolute alcohol. Dehydrated yogurt samples were freeze-dried using a freezer dryer (Alpha1-2, Christ Ltd., Vaihingen, Germany). Microstructure of yogurt samples were observed using scanning electron microscopy (JSM-6700F, Jeol Ltd., Tokyo, Japan).

Sensory Evaluation

Sensory evaluation was conducted by 12 panelists (10 female, 2 male), 20 to 30 yr old, recruited from the Lab-

oratory of Food Science Department, Jilin University (China). The sensory profile was established by using quantitative descriptive analysis (Gonzalez et al., 2011; Kaaki et al., 2012). All the panelists were selected after completing training according to ISO (1993) standards. The indicators of identity and quantity were evaluated using the reference properties as shown in Table 1. For all formulations, odor was the first property assessed and then the visual, flavor, and texture properties were evaluated. The intensity of the attributes was assessed using an evaluation sheet. Then 50 mL of the samples from the original containers were served at 4°C in random order under white light. The acceptability of the formulations were evaluated by 12 assessors of the products (Drake, 2007; Cruz et al., 2012) with a hedonic scale of 15 points (15 = very evident; 0 = none).

Statistical Analysis

All experiments were performed in triplicate. The results were expressed as mean ± standard deviation. The data were analyzed by SPSS version 11.5 (SPSS Inc., Chicago, IL). The significance level was set at $P < 0.05$ and $P < 0.01$. All the figures were drawn by Origin 8.0 (OriginLab Corporation, Northampton, MA).

RESULTS AND DISCUSSION

Preliminary Results

The effects of PWPS prepared through different treatment conditions (pH, heating temperature, heating time) on the viscosity of yogurt samples (YWPS) are shown in Figure 2. The results showed that yogurt adding PWP prepared at different pH, heating temperatures, and time had higher viscosity than that of yogurt without PWP. Heating the whey protein solution under controlled conditions to form a high molecular weight soluble whey protein aggregate results in an increase in viscosity (Vardhanabhuti and Foegeding, 1999). Figure 2A shows the yogurt samples contained PWP at different pH (7.0–9.0). No significant difference was observed in the viscosity of the yogurt sample with the addition of PWPS prepared between pH 7.0 and 8.0 ($P > 0.05$). The viscosity of yogurt with the addition of PWPS prepared at pH 8.5 and 9.0 was significantly higher than the control yogurt sample ($P < 0.01$). The increasing in the viscosity of yogurt was due to the larger volume of casein micelles caused by the interaction between PWP and casein micelles (Dybowska, 2011). The viscosity tends to increase with the increase of heating temperature, which may be due to protein aggregation caused by protein unfolding, aggregation, or both (Dybowska, 2011). When the heating temperature reached 75°C, the viscosity was at a peak of $3,683.33 \pm 86.23$ mPa·s and was significantly different from that of other samples ($P < 0.05$; Figure 2B). Figure 2C shows that the relationship between heating time of PWP and viscosity of yogurts. No significant difference was observed in viscosity of yogurts added with PWP prepared by different heating time range from 20 to 60 min ($P > 0.05$). However, the viscosity of yogurt with heating time as 10 min was significantly different from that of other yogurt samples ($P < 0.05$). Therefore, we selected 20 min as the optimal heating time. It has been reported that the viscosity of yogurt could be increased by the addition of PWP probably due to high level of cross-linking within the gel network during coagulation (Matumoto-Pintro et al., 2011).

Physicochemical Properties

The chemical composition of the experimental yogurt samples is shown in Table 2. The main reason for the decrease of pH during fermentation of yogurt was the lactic acid produced by *Lactobacillus* (Kailasapathy, 2006). The variation in pH and titratable acidity (TA) of yogurt samples may be due to the fact that the starter promotes hydrolysis of lactose in milk during fermentation (Wang et al., 2012). No significant difference was

Figure 2. Effects of polymerized whey protein solution (PWPS) prepared through different treatment conditions (pH, heating temperature, and heating time) on the viscosity of yogurt samples. The PWPS were prepared at 75°C for 20 min at different pH values (A), at different temperatures for 20 min at pH 7.0 (B), and at 75°C for different times at pH 7.0 (C). Means in the graph indicated by different letters (a–d) are significantly different ($P < 0.05$) from other treatment conditions. Error bars represent the standard deviation (SD) of the mean of triplicate experiments.

Table 2. Physicochemical properties of yogurt samples¹

Item	Control	YWPS	UNYWPS	YWPC
pH	4.27 ± 0.02 ^a	4.45 ± 0.03 ^b	4.32 ± 0.01 ^c	4.47 ± 0.03 ^b
Titrateable acidity (%)	87.49 ± 0.14 ^a	85.54 ± 0.31 ^b	91.35 ± 0.50 ^c	85.77 ± 0.39 ^b
TS (%)	11.88 ± 0.07 ^a	12.62 ± 0.34 ^b	12.23 ± 0.10 ^c	12.50 ± 0.37 ^b
Protein (%)	3.36 ± 0.08 ^a	4.29 ± 0.04 ^b	4.22 ± 0.04 ^{bc}	4.32 ± 0.03 ^{bd}
Fat (%)	3.97 ± 0.01 ^a	3.76 ± 0.02 ^b	3.55 ± 0.03 ^c	3.70 ± 0.05 ^b
Lactose (%)	4.33 ± 0.02	4.38 ± 0.11	4.37 ± 0.02	4.29 ± 0.01
Ash (%)	0.67 ± 0.02 ^a	0.73 ± 0.01 ^b	0.75 ± 0.01 ^b	0.76 ± 0.01 ^b

^{a-d}Different lowercase letters within each row indicate a significant difference ($P < 0.05$) between yogurts.

¹Values are means and SD of 2 replicates. Control = full-fat yogurt; YWPS = yogurt with liquid polymerized whey protein (heated at 75°C for 20 min, pH 7.0); UNYWPS = yogurt with unheated liquid whey protein; YWPC = yogurt with heat-treated whey protein concentrate (heated at 80°C for 30 min, pH 7.0).

observed in final pH value of YWPS and YWPC ($P > 0.05$), which were higher ($P < 0.05$) than those of UNYWPS samples and control, indicative of a good yogurt (Bensmira and Jiang, 2012). The differences in TA were not significant between YWPS and YWPC ($P > 0.05$), but the above 2 types of yogurt are lower than UNYWPS and control yogurt samples, and there was a significant difference between UNYWPS and control yogurt ($P < 0.01$). The TA values of yogurts may be associated with the nature of whey protein (heated or

unheated), addition amounts, and pH values. The differences in TA were not significant between YWPS and YWPC ($P > 0.05$), but the above 2 types of yogurt are lower than UNYWPS and control yogurt samples, and a significant difference was observed between UNYWPS and control yogurt ($P < 0.01$). However, the TS content of the control sample was lower than that of the other 3 yogurt samples. The protein and fat contents in all variations ranged between 3.3 and 4.3 g per 100 g, and 3.5 and 4.0 g per 100 g, respectively. The ash content of

Figure 3. Comparison of yogurts prepared using different polymerized whey protein for hardness (A), adhesiveness (B), springiness (C), cohesiveness (D), viscosity (E), and syneresis (F) of yogurt. Bars with different letters (a–c) are significantly different ($P < 0.05$). Control = full-fat yogurt; YWPS = yogurt with addition of liquid polymerized whey protein (heated at 75°C for 20 min, pH 7.0); UNYWPS = yogurt with unheated whey protein solids from cheese whey; YWPC = yogurt with addition of heat-treated whey protein concentrate (heated at 80°C for 30 min, pH 7.0). Means in the graph indicated by different letters (a–c) are significantly different ($P < 0.05$) from other treatment conditions. Error bars represent the standard deviation (SD) of the mean of triplicate experiments.

yogurt contained different types of thickening agent in an amount significantly higher than the control yogurt sample ($P < 0.05$).

Texture Profile and Viscosity

Texture is one of the most essential indexes of yogurt quality (Sodini et al., 2004). The hardness, adhesiveness, springiness, cohesiveness, and viscosity of set yogurt are listed in Figure 3. Significant differences were observed in the textural properties of different yogurt samples ($P < 0.05$); YWPS, YWPC, and UNYWPS had thicker and tighter texture than the control yogurt. Incorporation of heat-treated WPS significantly ($P < 0.05$) increased the adhesiveness, hardness, and springiness, whereas addition of unheated WPS did not significantly affect the texture properties compared with the heat-treated WPC. Yogurt samples with the addition of PWP exhibited higher hardness (Figure 3A), adhesiveness (Figure 3B), and springiness values (Figure 3C) than control yogurt ($P < 0.01$). There was no significant difference in hardness, adhesiveness, and springiness values between YWPS and YWPC ($P >$

0.05). The YWPS had significant values in hardness, adhesiveness, and springiness values than UNYWPS ($P < 0.01$). Yogurt samples with addition of PWP showed lower cohesiveness values than control yogurt ($P < 0.01$; Figure 3D). However, the control yogurts showed higher values of cohesiveness than other samples and were shown to be significantly different from other yogurt samples ($P < 0.01$; Figure 3).

Compared with control, addition of whey proteins (WPS, PWPS, and PWPC) significantly increased the viscosity of the yogurt. The viscosity of YWPS was significantly higher than UNYWPS ($P < 0.05$). No significant difference was observed in viscosity between YWPS and YWPC ($P > 0.05$). The heat-treated WPS and WPC led to the formation of aggregated protein and influenced viscosity of yogurt, resulting in firmer structures. The viscosity of PWP was much higher than unheated whey protein solids, possibly due to the low molecular weight of native whey protein. The whey protein was heated to form a high molecular weight whey protein polymer similar to hydrocolloids and used as thickening agents in food products, similar to a previous study (Vardhanabhuti et al., 2001). The addition

Figure 4. Scanning electron micrographs of 4 yogurt samples: (A) control = full-fat yogurt; (B) YWPS = yogurt with addition of liquid polymerized whey protein (heated at 75°C for 20 min, pH 7.0); (C) UNYWPS = yogurt with addition of unheated whey protein solids from cheese whey; and (D) YWPC = yogurt with addition of heat-treated whey protein concentrate (heated at 80°C for 30 min, pH 7.0).

of PWP in yogurt making not only affects coagulation time but also the level of syneresis (Schorsch et al., 2001).

Syneresis

Syneresis is one of the important indexes to evaluate the quality of yogurt and is the major visible defect that occurs during yogurt storage (Ghorbanzade et al., 2017; Vianna et al., 2017). In Figure 3D, the results revealed that syneresis of the yogurt with heat-treated WPS directly obtained by cheese whey yogurts (YWPS) was similar to that of YWPC ($P > 0.05$). However, the syneresis of the samples with PWP was lower than UNYWPC ($P < 0.05$), and there was significant difference with control yogurt ($P < 0.01$). The yogurt with unheated WPS had higher syneresis. This may be due to the protein-water interactions resulting in a weaker texture of yogurt with the addition

of unheated WPS (Cheng et al., 2017). The control yogurt showed a higher value of syneresis than other samples ($P < 0.01$). Similar to Britten's (de Wit, 1989) theory, our results demonstrate that the addition of PWP to yogurt reduced syneresis of yogurt. However, Schorsch et al. (2001) showed that the gel formed by heat-treated whey protein and casein micelles was more likely to produce syneresis. The contradiction of the syneresis could be due to the different gel system, PWP manufacture conditions, and storage temperature. Because the whey protein gel has strong water-retaining capacity and PWP has the characteristics of an acid-induced cold gel, yogurt with added PWP can maintain the stability of the water phase in the yogurt network, thus reducing its susceptibility to syneresis (Li and Guo, 2006). On the other hand, from the structure of the yogurt shown in the micrographs (Figure 4), PWP adsorption on the surface of the casein micelles forms casein/PWP complexes, making the protein networks

Figure 5. Sensory results from quantitative descriptive analysis from 4 yogurt samples: control = full-fat yogurt; YWPS = yogurt prepared with polymerized whey protein solids (heated at 75°C for 20 min, pH 7.0) from cheese whey; UNYWPS = yogurt with unheated whey protein solids from cheese whey; YWPC = yogurt with heat-treated whey protein concentrate (heated at 80°C for 30 min, pH 7.0) of 14 major attributes. T = texture; V = visual; O = odor; F = flavor.

of the yogurt more uniform, which may cause the synergistic effect network to weaken with the decrease of pore size (Farnsworth et al., 2006).

Microstructure

Scanning electron micrographs of yogurt samples are shown in Figure 4: control (A), YWPS (B), UNYWPS (C), and YWPC (D). The scanning electron micrograph revealed that the distribution of casein micelles was relatively uniform and the size was relatively similar. In comparison with control yogurt (Figure 4A), yogurt samples with addition of whey proteins, such as YWPS (Figure 4B), UNYWPS (Figure 4C), and YWPC (Figure 4D), formed larger and denser protein clusters. A previous study found that addition of preheated whey proteins to casein micelles resulted in formation of bridges between the casein particles by binding with denatured whey protein to the surface of micelle during acidification (Schorsch et al., 2001). Scanning electron microscopy analysis of the microstructure of yogurt showed that the yogurt with denatured WPS formed a more comprehensive network structure that improved the water retention ability. Differences were observed in texture and microstructure of yogurt with different types of stabilizers, which may be due to the different stabilization mechanisms of different thickeners, thus

affecting the electrostatic interactions between protein molecules in the network. The direct interaction between casein micelle and denatured whey protein was observed from Figure 4, probably because the interaction between casein micelle and PWP mainly leads to the formation of casein-PWP complex through hydrophobic action. The combination of PWP and micelle surface helps to form a bridge between casein particles and form a network structure, which improves the consistency and increases the water retention of yogurt.

Sensory Property

A selected yogurt sample (YWPS) with added PWP was evaluated by sensory analysis in comparison to the control yogurt, UNYWPS, and YWPC. Each of the yogurt samples was tested for the 14 major sensory attributes (Table 1) and the sensory scores were presented in the spider diagram in Figure 5 (Nguyen et al., 2017). The intensity of each characteristic expressed by different yogurt samples in the same sensory properties was different. However, no significant difference ($P > 0.05$) was observed between the YWPC and YWPS in sensory evaluation indexes. These 2 kinds of yogurt were compared with UNYWPS having differences ($P < 0.05$) in various indicators and being significantly higher ($P < 0.01$) than the control sample in sensory

Figure 6. Principal component (PC) analysis biplots of the 4 yogurt samples including PC1 and PC3, and PC1 and PC2. Identification of samples is given in Table 1. T = texture; V = visual; O = odor; F = flavor.

evaluation indexes except for texture (**T**)-decentrality. This may be due to the higher viscosity and lower syneresis of the yogurt with added thickening agents (YWPC and YWPS), and sensory evaluation results have been consistent with the results shown in the Figure 3. Some studies have shown that the assessors after training were correlated positively with food preferences although sensory measurements are still subjective (Lopez-Enriquez et al., 2015; Tahsiri et al., 2017).

Principal component analysis was performed on sensory scores for 4 yogurt samples and the 14 attributes as shown in Table 1. The principal component analysis biplot of the yogurt samples (Figure 6) illustrates specific differences among the products. The biplot explains 87.42% of the total variation as shown in Figure 6. The first 2 principal components accounted for 52.3% of the variability in the results. The main loading factor included visual (**V**)-syneresis, V-thickness, flavor (**F**)-sweetness, T-mouthcoating, and T-smoothness. The second principal components accounted for 24.32% of the variability in the results, which included T-decentrality, V-homogeneity, odor (**O**)-sweetness, and F-acidity. The third factor on the y-axis explaining 10.8% is shown on the 3-dimensional diagram of Figure 6. There was similar distribution between the YWPC and YWPS in the positive part of the first dimension, and they were similar to each other in visual, odor, flavor, and texture aspects mentioned in Table 1, and the sensory characteristics such as T-mouthcoating, T-smoothness, and V-syneresis were particularly obvious. Attribute vectors for T-decentrality, V-homogeneity, O-sweetness, and F-acidity form the second principal dimension. In terms of T-decentrality, the 4 yogurt samples could be divided into 2 groups: lower decentrality (YWPC and YWPS) and higher decentrality (control and UNYWPS). The control yogurt sample was similar to UNYWPS in sensory index, but the 2 yogurt samples mentioned above were significantly different from YWPC and YWPS. It may be that the effect of viscosity of yogurt was related to adding a variety of polymeric whey proteins as the thickening agent, resulting in different decentrality of yogurt samples and affecting sensory evaluation of yogurt (Tahsiri et al., 2017).

CONCLUSIONS

Polymerized liquid whey protein concentrate prepared directly from Cheddar cheese whey can be used as a thickening agent for full-fat yogurt formulation. This procedure provides an alternative way to prepare PWP obtained through thermal aggregation of conventional dry products, such as WPC or WPI, in manufacturing these dairy products.

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