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Effects of Encapsulated Fish Oil by Polymerized Whey Protein on the Textural and Sensory Characteristics of Low-Fat Yogurt

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Five types of polymerized whey protein (PWP1, PWP2, PWP3, PWP4 and PWP5) containing different amounts of fish oil were added to low-fat yogurt as fat replacers. The texture, apparent viscosity, and sensory properties of the yogurts were analyzed in comparison with full-fat (3.0%, w/w, fat) and low-fat (1.5%, w/w; and 1.2%, w/w) milk yogurt controls. The majority (~85%) of the particle size distribution was in the range of 1106±158 nm. Thermal property analysis indicated PWP was thermally stable between 50°C and 90°C. Yogurts formulated with 12% of PWP4 and 14% of PWP5 demonstrated higher firmness, springiness and adhesiveness ($P<0.05$), and lower cohesiveness ($P<0.05$) than the low-fat milk yogurt controls. There was no fat separation and they had less fishy smell. Yogurts incorporated with 12% of PWP4 had comparable sensory and textural characteristics to the full-fat milk yogurt control.

INTRODUCTION

Yogurt has many nutraceutical or therapeutic effects such as enhancing digestion and immune systems and reducing serum cholesterol, as well as anticarcinogenic activity [Bertolami *et al.*, 1999; Shah, 2001; Sloan, 2001; Milo-Ohr, 2002]. Low-fat yogurt is gaining more and more interests because it contains less calories than full-fat yogurt while preserves the majority of nutraceutical nutrients. However, the flavor of low-fat yogurt is unbalanced due to its low-fat content [Janhoj & Ipsen, 2006; Torres *et al.*, 2011].

Milk fat acts as a carrier for fat-soluble flavors and nutrients [Brauss *et al.*, 1999; Zhang *et al.*, 2015], and it also affects the texture of yogurt, such as gelation and syneresis [Lucey, 2004], thus milk fat is important for organoleptic characteristics, *e.g.*, gloss, color and taste [Guven *et al.*, 2005]. Modler & Kalab [1983], Akalin *et al.* [2012] and Andoyo *et al.* [2014] reported that casein micelles in skim milk yogurt stabilized with whey protein concentrates in the form of individual entities were surrounded by finely flocculated protein. Alting *et al.* [2009] improved creaminess of low-fat yogurt by using amylomaltase-treated starch. Srisuvor *et al.* [2013] used inulin and polydextrose as a fat replacer in low-fat yogurt to improve sensory properties. Komatsu *et al.* [2013] produced a functional guava mousses formulated with inulin and whey protein concentrate to partially or totally substitute the milk fat content.

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are polyunsaturated fatty acids and belong to ω-3 fatty acid, which are mainly found in fish oil. A relative dietary ω-3 fatty acid deficiency may be associated with some chronic diseases because of the importance of ω-3 fatty acids in essential cell characteristics and functions such as membrane fluidity, cellular signaling, gene expression, and eicosanoid metabolism [McCowen & Bistrian, 2005]. McCowen *et al.* [2010] developed a stable emulsion of DHA that was incorporated into yogurt, and found that fortification of yogurt with DHA is a potentially attractive method of increasing ω-3 fatty acid content of plasma lipids. In order to cover up the fishy smell of the fish oil in the yogurt, Estrada *et al.* [2011] put strawberry puree into milk fermented with fish oil, but in our study, we made fish oil microencapsulated in polymerized whey protein (PWP) to protect it from oxidization and reduce the unpleasant fishy smell. For an ideal fat replacer, it should be natural, resourceful, low-cost, and suitable for industrial-scale production. PWP was prepared from whey protein concentrate (WPC 80) by thermal treatment [Zhang *et al.*, 2013]. The objectives of this study were to develop a low-fat yogurt using PWP as a fat replacer and investigate the effects of PWP containing fish oil on textural characteristic and sensory evaluation of low-fat yogurt.

MATERIALS AND METHODS

Preparation of polymerized whey protein (PWP)

Whey protein concentrate 80 powder (Fonterra Co-operative Group, New Zealand) was reconstituted at a pro-

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TABLE 1. Formulations of polymerized whey protein with and without fish oil.

	WPC 80 (g/100 g)	DHA+EPA (mg/100 g)
PWP0	10	0
PWP 1	10	2200
PWP 2	10	1900
PWP 3	10	1600
PWP 4	10	1300
PWP 5	10	1000

Yogurt A to E (TABLE 2) and yogurt 1 to 7 (TABLE 3) were supplied with fish oil (DHA+EPA=216.67 mg) which met 1/3 of the recommended amount (650 mg /2000 kcals, which is recommended by International Society for the study of Fatty Acids and Lipids) by adding with a certain ratio of varying types of PWP (PWP1 to PWP5).

tein concentration of 100 g/L by dissolving it into distilled water slowly with a continuously stir, and then stored overnight at 4°C for a better hydration. Fish oil (containing DHA 183 mg/g, EPA 129 mg/g, Changchun Health Food Co., Ltd, China) was added to the WPC solution and then homogenized at 10 MPa or 20 MPa using a homogenizer (Langfang General Machinery Co., Ltd, China). The formulations were listed in Table 1. The dispersion was warmed up to room temperature and its pH was adjusted to 8.5 with 2 mol/L sodium hydroxide before polymerized at 85°C for 30 min.

Emulsifying activity index (EAI)

EAI of PWP was determined by using the modified method described by Pearce & Kinsella [1978]. The emulsions (25°C) were first stirred by using a high-speed dispersing and emulsifying unit (Model IKA-ULTRA-TURRAX-T25 basic, IKA, Works, Inc., Wilmington, North Carolina, USA) at 21,500 rpm for 2 min. The sheared (o/w) emulsions (100 μL) were then diluted with 5 mL of 0.1 mol/L phosphate buffer containing 1 g/L sodium dodecyl sulfate (SDS). The absorbance at 500 nm of the diluted emulsions was then determined by using a spectrophotometer (Model UV-2550, Shimadzu Corporation, Japan). The emulsifying activity index (EAI) was calculated as:

$$\mathrm{EAI}\ (\mathrm{m^2 g^{-1}}) = \frac{2 \times 2.303 \times \mathrm{A} \times \mathrm{D}}{\mathrm{L} \times \text{Ø} \times \mathrm{C} \times 10^4} \tag{1}$$

where A is the absorbance at 500 nm, and L is the path length of the cuvette (1 cm), D is the dilution factor, Ø is the volumetric fraction of oil, C is the mass of protein per unit volume of aqueous phase before the emulsion was formed (g/mL) and 10^4 is the correction factor for square meters. The EAI of the emulsions held at 4°C for 24 h and 48 h were determined.

Thermal analysis of PWP

Thermal properties of PWP were determined using a differential scanning calorimeter (DSC Q2000, TA Instrument-Waters LLC, USA) according to the method described by Fitzsimons *et al.* [2007]. The instrument was calibrated using indium (T_{peak} = 155.87 °C, DH = 28.234 Jg^{-1}) and zinc (T_{peak} =417.4 °C, ΔH = 93.337 J g^{-1}). The PWP (18.8 mg) were added into stainless steel pans. An empty pan of equal weight served as the reference and all pans were hermetically sealed before placing in the instrument. The samples were scanned from 30 to 100°C at a scanning rate of 1.0°C /min.

Analysis of polymerized whey protein particle size

The particle size (diameter) distribution of polymerized whey protein was determined by a laser diffraction particle size analyzer ZETA SIZER Nano-zs (Malvern Instruments, Malvern, England, UK). Before taking the measurements, the PWP samples were diluted with ultrapure water and under constantly stirring, which made the PWP transparent particles suspension liquid [Xie *et al.*, 2007]. The particle size distribution and average particle size were analyzed in triplicate.

Determination of the amount of PWP addition as a fat replacer

Skimmed milk (1%, w/w, fat) was preheated to 40°C, and different amounts and types of PWP were added to skimmed milk with moderate stirring (Table 2). Then the mixture was homogenized (10 MPa, once), pasteurized (85°C for 20 min), cooled to 43°C and inoculated with a yogurt starter culture composed of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus thermophilus* (F-DVS YF-3331; Chr. Hansen A/S, Denmark). The starter culture was selected based on its low ability to produce exopolysaccharides, and thereby ensured a minimal influence of the starter culture on the texture of the yogurt [Torres *et al.*, 2011]. The fermentation procedure was ceased by putting the sample into a refrigerator until the pH dropped to 4.5.

Commercially available full-fat milk (3%, w/w, fat), skimmed milk (0.1%, w/w, fat) and their mixture were used to make yogurt samples for comparison with low-fat yogurts with fat replacer. The formulations were shown in Table 3, and the preparation of yogurt was the same as described above.

Analyses of physiochemical properties

Texture

Yogurt texture analyses were carried out by using a Texture Analyzer (Brookfield Engineering Labs, Inc., New York) with two-cycle comparison procedure by a 1 kg load cell.

TABLE 2. Formulations of low-fat yogurt A to E with fat replacer PWP 1 to 5.

Yogurt types	Skimmed milk/g	FR types	FR ratio/%
A	140.7	PWP1	6
B	137.7	PWP 2	8
C	134.7	PWP 3	10
D	131.7	PWP 4	12
E	128.7	PWP 5	14

Mass of non-fat yogurt A to E was 150 g per container. Each sample enriched with the same amount of fish oil (DHA + EPA = 216.67 mg).

TABLE 3. The formulations of different types of yogurts.

Yogurt code	Skimmed milk (g)	Full-fat milk (g)	WPC (g)	FR types	FR ratio/% (w/w)	Fish oil
1	131.7	—	—	PWP4	12	Encapsulated
2	128.7	—	—	PWP5	14	Encapsulated
3	131.8	—	—	PWP0	12	Added directly
4	131.8	—	17.97	—	—	Added directly
5	—	150	—	—	—	—
6	75	75	—	—	—	—
7	112.5	37.5	—	—	—	—

Yogurt 1 to 4, low-fat; yogurt 5, full-fat; yogurt 6, semi-skimmed; yogurt 7, 75% skimmed. Yogurt 4 was supplemented with 12% (w/w) WPC80 dispersions (10%, w/v). Mass of yogurt 1 to 7 was 150 g per container. Yogurts with FR enriched with the same amount of fish oil (DHA + EPA = 216.67 mg).

The crosshead velocity was set at 1 mms^{-1}, and sample depth was 30 mm. The record began when the detector surface was in full contact with the yogurt with surface trigger of 4.5 g. As defined by Brennan [1984] and Nishinari *et al.* [2013], the value of springiness (the height that the sample recovers between the end of the first cycle and the start of the second cycle), adhesiveness (the negative force area for the first cycle, representing the work necessary to pull the compressing plunger away from the sample), and cohesiveness (the ratio of the positive force area during the second compression to that during the first compression) were calculated. The maximum force as the test cell penetrated 30 mm into the sample as described by Damin *et al.* [2008] was regarded as the firmness of the sample. All tests were carried out in triplicates.

Apparent viscosity

The yogurts were stirred for around 10 laps with a glass rod before being measured. The apparent viscosity of yogurt was measured after fermentation using a dynamic viscometer (Brookfield Model-LV; Brookfield Engineering Laboratory, Stoughton, USA) at a speed of 100 rpm. All the assays were performed in triplicate.

Sensory analysis

Sensory evaluation was carried out after the yogurt samples were stored at 4–6°C for 24 h by a ten-member panel. The volunteers for the sensory evaluation were between the ages of 20 and 50, approximately balanced between females and males, moreover, they were informed that some of the samples were containing fish oil. Each panelist was given 7 yogurt samples (samples 1～7), which were presented in a random order. We made a brief explanation about each of the sensory attribute, and all the panelists had to score visual features (whey separation, floating lipid, smoothness, glossiness), and gustatory features (firmness, palatability, creaminess, fishy smell, viscosity, acceptability) on a hedonic scale of 1 (very bad) to 10 (excellent) [Ding *et al.*, 2011].

Statistical analysis

All analyses and enumerations were done in triplicate. Analysis of variance (ANOVA) was applied on emulsifying activity index, texture, apparent viscosity data, and sensory analysis using the SPSS11.5 program. Differences among means were tested for significance by Duncan's multiple range test. The level of significance was set at 95%.

RESULTS AND DISCUSSION

Effects of heating time and homogenization pressure on EAI of PWP

The emulsifying activity index (EAI) is related to the surface area stabilized by a unit mass of proteins. Some studies have shown that fat droplets may bind to β-lactoglobulin (the first major protein in WPC) at two different sites. The first site is the central calyx located on the β-sheet strands where most of the hydrophobic groups are located and the other is on the protein surface itself [Loch *et al.*, 2011]. Heat treatment and homogenization may help α-lactalbumin (the second major protein in WPC) obtain a more open structure due to electrostatic repulsive charges, which may help proteins to spread out across a fat droplet surface and enhances its droplet coverage [Zhai *et al.*, 2012; Lam & Nickerson, 2015a]. EAI represents the ability of proteins to be adsorbed at the interface of fat globules and the aqueous phase [Pearce & Kinsella, 1978]. The results shown in Figure 1A revealed that EAI significantly ($p<0.05$) increased by 98% from the minimum 76 m^2 g^{-1} (PWP modified without homogenization, and was held for 20 min at 85°C) to the maximum 150.5 m^2 g^{-1} (PWP modified after homogenization in 20 MPa, and was held for 30min at 85°C). But there was no significant difference in EAI between homogenization at 10 MPa and 20 MPa ($p>0.05$). It is possible that changes in whey protein structure caused by thermal polymerization lead to an increased surface hydrophobicity and molecular flexibility, allowing an effective adsorption of protein molecules at the oil–water interface [Manoi & Rizvi, 2009]. It is well documented that the polymerized proteins usually exhibit a high surface hydrophobicity which enhances emulsifying activity and interfacial concentration by contributing to the film rigidity through hydrophobic interactions between adjacent protein molecules at the interface [Mitidieri & Wagner, 2002; Guilmineau & Kulozik, 2007; Sullivan *et al.*, 2008; Kang *et al.*, 2014; Perez *et al.*, 2014; Segat *et al.*, 2014].

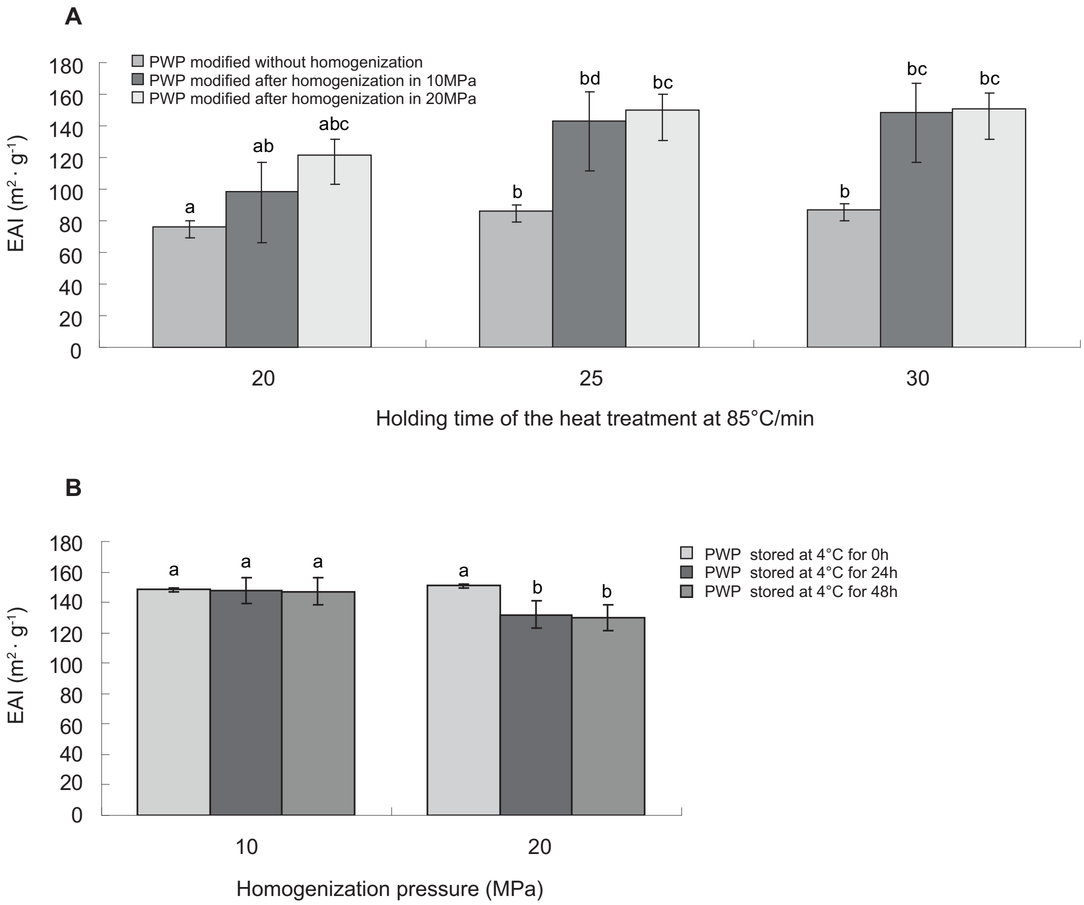

FIGURE 1. Effects of heating time and homogenization pressure on emulsifying activity index of polymerized whey protein. Values with different superscript letters are significantly different ($p<0.05$).

The results shown in Figure 1B revealed that over storage of 24 h, the EAI of PWP modified after homogenization at 20 MPa decreased ($p<0.05$), whereas that of PWP modified after homogenization at 10 MPa remained unchanged and both of them stayed steady from 24 h to 48 h at 4°C. During this process of homogenization, the combination of intense shear, cavitation and turbulent flow conditions leads to disruption of the fat droplets, meanwhile, whey proteins play the role of emulsifiers, which adsorb to the surface of fat droplets, and form a protective layer that prevents the droplets from aggregating [Lam & Nickerson, 2015b]. The decrease in average size of the fat droplets is due to the increase of homogenization pressure and improvement of emulsifying ability. However, increase of the pressure of homogenization is related to the increase of the temperature of emulsions, therefore, 20 MPa of homogenization pressure will improve the temperature in a greater degree than 10 MPa. As we know, proteins are sensitive to heat, which could make emulsions tend to break down over time due to protein unfolding exposure [Bernard *et al.*, 2011] and interaction between hydrophobic groups *via* the formation of covalent bonds. All these changes may be caused by the increased temperature, thereby resulting in promoting droplet coalescence and a decrease of emulsifying stability (Figure 1B, EAI value of PWP under the homogenization pressure of 20 MPa dropped at 24 h and 48 h). In conclusion, homogenization at 10 MPa is more suitable for PWP to retain fish oil.

Thermal analysis of polymerized whey protein

Figure 2 showed DSC curve recorded (at a heating rate of 1.0°C/min) for PWP at pH 8.5. The physicochemical properties of whey proteins present as well as intrinsic (structure and conformation) including Van der Waals and steric forces, different attractive or repulsive molecular forces, and extrinsic (environment) conditions including pH, temperature, ionic strength, solvent polarity and type, govern the whey protein functionality [Dissanayake *et al.*, 2013].

Purwanti *et al.* [2011] prepared soluble aggregates by heating 3% or 9% whey protein isolate (WPI) solutions at 90°C for 30 min. Heat treatment makes soluble whey protein aggregates formed at concentrations (10%, w/w, in our study) below their critical gelation concentration, which is approximately 12% [Purwanti *et al.*, 2011]. The soluble aggregates should be small and roughly spherical, have high surface charge, and low surface hydrophobicity for maximum thermal stability [Wijayanti *et al.*, 2014]. The enhanced thermal stability of PWP may be due to their higher overall negative charge, more compact structure with less branching, and small size which make them resistant to secondary interactions [Ryan *et al.*, 2012; Wijayanti *et al.*, 2014]. Unfolding of globular proteins during thermal denaturation involves absorption of heat to break intramolecular bonds (non-covalent and, in some cases, disulfide) and is therefore endothermic [Fitzsimons *et al.*, 2007]. Aggregation of the denatured molecules involves formation of new intermolecular bonds, and would therefore

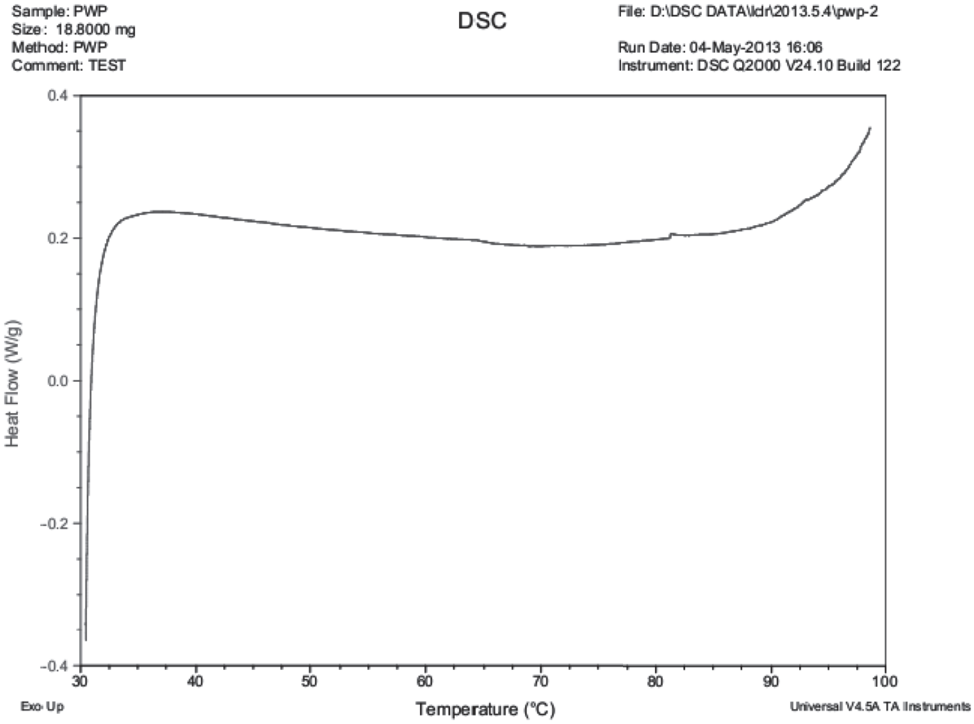

FIGURE 2. DSC heating scans (1.0°C/min) of polymerized whey protein at pH 8.5.

be expected to give rise to an exothermic process in DSC. One way of promoting aggregation and gelation is to reduce the charge on the protein molecules by lowering the pH towards the isoelectric point [Boye & Alli, 2000]. Aggregation can, however, also be promoted by the addition of salt to screen the intermolecular repulsions [Varunsatian *et al.*, 1983; Verheul *et al.*, 1995; Puyol *et al.*, 2001; Abhyankar *et al.*, 2014]. Ryan *et al.* [2012] found soluble aggregates formed from whey protein were more thermally stable in solutions with salt solution. On the contrary, at a neutral or high pH in the absence of added salt, the aggregation process is inhibited by electrostatic repulsion between the globules.

It was found that neither an endothermic nor an exothermic curve but a line similarly horizontal between 50 and 90°C, which meant the PWP was mostly polymerized and barely affected by heat from 50 ~ 90°C, and such a conclusion was in accordance with the previous research [Purwanti *et al.*, 2011]. Therefore, the PWP may have thermal stabilities in this temperature range, and the DSC curve provides the sterilization temperature range (lower than 90°C) for low-fat yogurt containing PWP as a fat replacer.

Polymerized whey protein particle size

Figure 3 shows the particle size distribution by mass of the PWP. The size distribution curves showed a multimodal distribution because of the different extent of polymerization. This overall sample polydispersity, derived from polydispersity index (PdI), is polydisperse, so average particle size (Table 4) is skewed toward lager values, and suggest rely on distribution analysis sizes (Figure 3). When the PdI value was between 0.08 and 0.7, which indicates that the sample has a moderate dispersion, it was the best for particle size measurement. Only when the sample has a single component (PdI<0.05), spherical and monodispersity, the average particle size is reliable. The test report provided by Malvern company suggests that there are a number of populations within the sample or a significant proportion of aggregated material, and such a conclusion is in conformity with the PWP characteristics. The particle quantity of whose size in the range of 1106±158 nm is 84.8%, the remaining 15.2% of the particle size is between 190.1±119 nm (Figure 3). A small particle size has a relatively large surface area and high surface energies, which was very prone to spontaneous aggregation. The PWP remain soluble and their properties, such as aggregate size, keep constant for several days [Floris *et al.*, 2008]. Fat globule usually exists in yogurt in average size of 1 μm [Yan *et al.*, 2012], and the majority

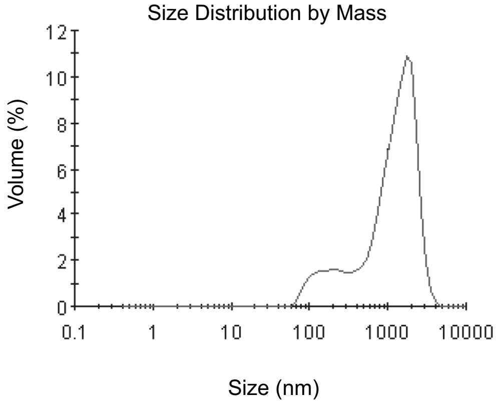

FIGURE 3. Particle size (diameter) distribution by mass of polymerized whey protein at 25°C.

TABLE 4. Average particle size of polymerized whey protein.

Record	T/°C	Z-Ave / d.nm	PdI	Mean Count Rate / kcps	Derived Count Rate / kcps	Intercept
1	25.00	266.60	0.50	197.30	139286.60	0.97
2	25.00	265.30	0.48	197.70	139527.20	0.97
3	25.00	263.30	0.48	196.40	138613.60	0.97
Mean	25.00	265.10	0.49	197.10	139142.50	0.97

of PWP particle size is close to 1 μm. The test results indicate that PWP may be suitable for a fat replacer.

Texture

Texture is one of the most essential components of yogurt quality and related to sensory perception of food product, thus it can represent all the rheological and structural attributes perceptible by means of mechanical, tactile, visual and auditory receptors [Sodini *et al.*, 2004]. The texture of yogurt gel is governed by the development of a three-dimensional network of milk proteins. The main factor responsible for yogurt is a reduction in high net negative charge on the casein micelles. During fermentation, casein micelles and PWP, aggregate into chains and clusters through hydrophobic and electrostatic bonds, affecting the structure of yogurt [Paseephol *et al.*, 2008]. The textural properties of low-fat yogurt A to E (produced by skimmed milk, and each sample was enriched with 216.67 mg fish oil *via* addition of PWP1 to PWP5) were presented in Figure 4. Firmness of yogurt was increased by 88% from 108.55 g in yogurt A to 204.27 g in yogurt E, and yogurt C had the lowest value of cohesiveness but no significant difference was found among all the yogurts (p>0.05). Cross-linking or bridging of PWP associated with the casein micelles results in an increase in the number and strength of bonds between pro-

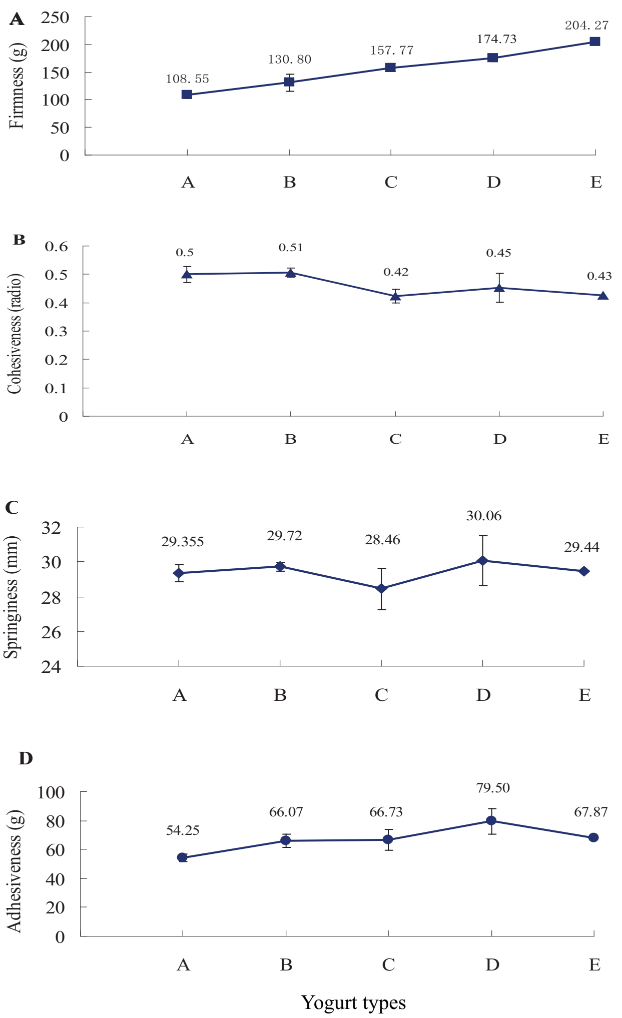

FIGURE 4. Textural properties of yogurts. Yogurt from skim milk + 6% PWP1, A; yogurt from skim milk + 8% PWP2, B; yogurt from skim milk + 10% PWP3, C; yogurt from skim milk + 12% PWP4, D; yogurt from skim milk+ 14% PWP5, E.

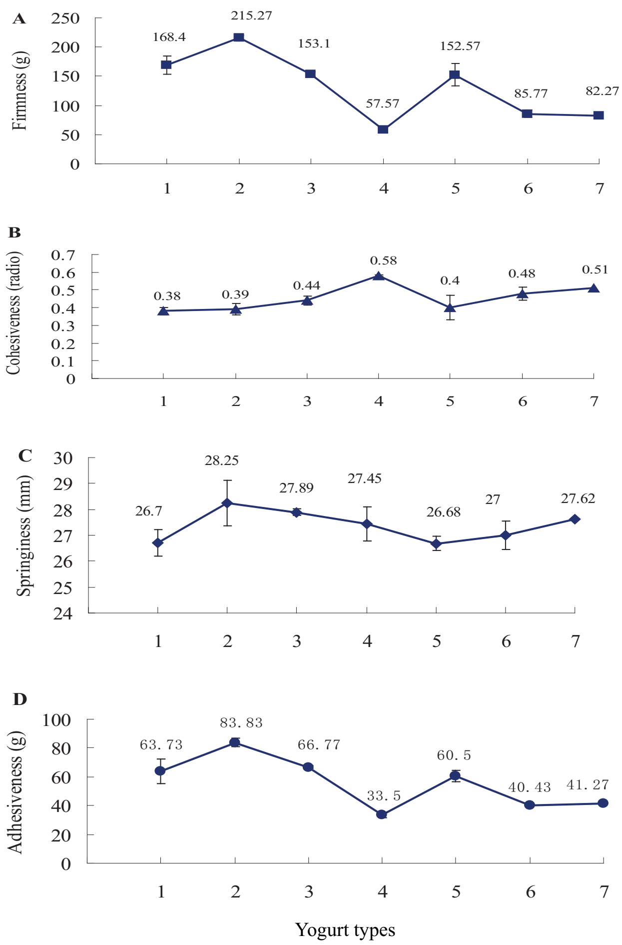

FIGURE 5. Textural properties of yogurts. Yogurt from skim milk + 12% PWP4 +microencapsulated fish oil, 1; yogurt from skim milk + 14% PWP5 + microencapsulated fish oil, 2; yogurt from skim milk + 12% PWP4 + fish oil, 3; yogurt from skim milk + 12% WPC+ fish oil, 4; yogurt from full-fat milk, 5; yogurt from semi-skimmed milk, 6; and yogurt from 75% skimmed milk, 7.

teins [Sodini *et al.*, 2004]. Higher protein content would cause a higher degree of cross-linkage of the gel network, resulting in a much denser and more rigid gel structure [Paseephol *et al.*, 2008]. This is mainly responsible for the significant increase of firmness. Yogurt D and E had higher values of springiness and adhesiveness than any others, but no significant difference between the two was observed. In this case, polymerized whey protein 4 (PWP4) and polymerized whey protein 5 (PWP5) were going to be added to low-fat yogurts and compared with full-fat yogurt, semi-skimmed yogurt, and 75% skimmed yogurt (Table 3). It is known that yogurt texture is highly dependent on total solids content as well as protein content and type [Oliveira *et al.*, 2001; Sandoval-Castilla *et al.*, 2004]. The addition of PWP to milk resulted in a more rigid gel structure in yogurt due to the formation of aggregates by interaction with casein micelles [Herrero & Requena, 2006].

Figure 5 shows that low-fat yogurt incorporated with PWP4 and PWP5 (types 1, 2 and 3) exhibited higher firmness, springiness and adhesiveness, but lower cohesiveness than the low-fat yogurt fortified with WPC (type 4) and other low-fat yogurts (types 6 and 7) ($p<0.05$). The lower cohesiveness the smoother yogurt texture, due to cohesiveness is related to the strength of the internal bonds in yogurt structure. Yogurt type 1 (microencapsulated fish oil) showed similar texture (firmness and adhesiveness) as that of low-fat yogurt type 3 (fish oil was added directly) but lower cohesiveness ($p<0.05$). That meant microencapsulated fish oil in PWP would provide a better uniform and smooth texture for yogurt.

The difference between types 1 and 2 was the amount of PWP. Type 2 showed higher firmness, springiness and adhesiveness ($p<0.05$), however, by taking the texture index of full-fat yogurt for reference, low-fat yogurt with 12% PWP4 had most similar texture as that of full-fat yogurt.

Apparent viscosity

Figure 6 shows results of apparent viscosity after manufacturing of fermented milk samples. In Figure 6 A, apparent viscosity increased by 15.2% from 936.9 mPa.s in yogurt A to 1079.9 mPa.s in yogurt E. According to the results of textural properties (Figure 4) and apparent viscosity (Figure 6 A), 12% of PWP4 and 14% of PWP5 used as fat replacers added to low-fat yogurt improved the apparent viscosity and textural properties which showed no signifi-

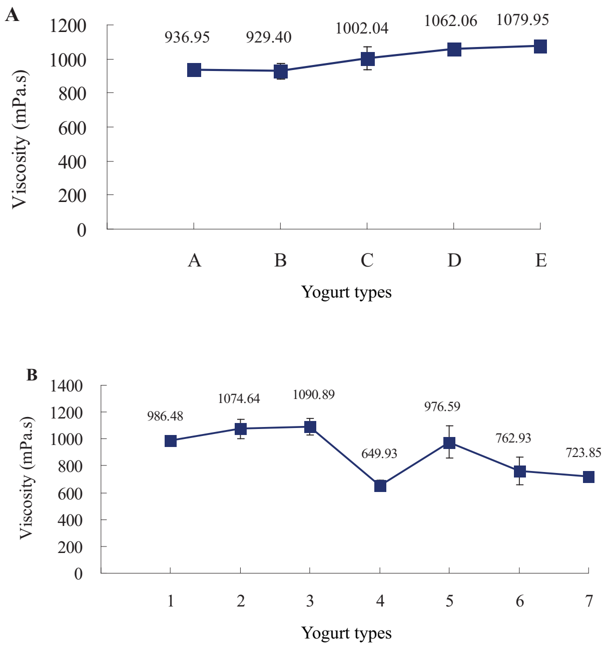

FIGURE 6. Apparent viscosity of yogurts. (A) Yogurt from skim milk + 6% PWP1, A; yogurt from skim milk + 8% PWP2, B; yogurt from skim milk + 10% PWP3, C; yogurt from skim milk + 12% PWP4, D; yogurt from skim milk+ 14% PWP5, E. (B) Yogurt from skim milk + 12% PWP4 +microencapsulated fish oil, 1; yogurt from skim milk + 14% PWP5 + microencapsulated fish oil, 2; yogurt from skim milk + 12% PWP4 + fish oil, 3; yogurt from skim milk + 12% WPC+ fish oil, 4; yogurt from full-fat milk, 5; yogurt from semi-skimmed milk, 6; and yogurt from 75% skimmed milk, 7.

TABLE 5. Mean value of indexes of sensory evaluation (mean ± standard deviation, n = 10).

Sensory attributes	Yogurt types	1	2	3	4	5	6	7
Visual features	whey separation	7.5 ± 0.6^a	7.9 ± 0.5^a	7.5 ± 0.9^a	6.8 ± 1.3^a	7.9 ± 0.2^a	5.3 ± 1.1^b	4.3 ± 0.6^b
	floating lipid	9.7 ± 0.3^a	9.9 ± 0.1^a	7.6 ± 1.1^b	7.1 ± 0.7^b	10 ± 0^a	10 ± 0^a	10 ± 0^a
	smoothness	9.6 ± 0.2^a	9.6 ± 0.3^a	9.2 ± 0.5^b	9.3 ± 0.2^a	9.7 ± 0.1^a	9.5 ± 0.1^a	9.5 ± 0.3^a
	glossiness	9.4 ± 0.2^a	9.4 ± 0.1^a	9.0 ± 0.5^a	9.2 ± 0.1^a	9.5 ± 0.1^a	9.3 ± 0.4^a	9.2 ± 0.7^a
Gustatory features	firmness	9.8 ± 0.1^a	9.8 ± 0.2^a	9.6 ± 0.1^a	9.1 ± 0.6^a	9.6 ± 0.2^a	8.5 ± 0.6^b	8.1 ± 0.2^b
	palatability	9.2 ± 0.3^a	9.0 ± 0.1^a	9.1 ± 0.2^a	8.7 ± 0.6^b	9.5 ± 0.1^a	8.5 ± 0.5^b	8.2 ± 0.4^b
	creaminess	9.5 ± 0.1^a	9.5 ± 0.4^a	9.4 ± 0.3^a	9.2 ± 0.1^a	9.5 ± 0.2^a	9.4 ± 0.3^a	9.4 ± 0.1^a
	fishy smell	8.5 ± 0.9^b	8.3 ± 1.2^b	5.6 ± 0.7^c	5.1 ± 0.2^c	10 ± 0^a	10 ± 0^a	10 ± 0^a
	viscosity	9.5 ± 0.1^a	9.6 ± 0.3^a	9.5 ± 0.2^a	9.1 ± 0.3^a	9.6 ± 0.2^a	8.5 ± 0.1^b	7.7 ± 0.6^b
	acceptability	9.1 ± 0.6^a	9.1 ± 0.4^a	8.4 ± 0.7^b	8.0 ± 0.6^b	9.4 ± 0.2^a	8.9 ± 0.4^a	8.0 ± 0.3^b

Yogurt from skim milk + 12% PWP4 +microencapsulated fish oil, 1; yogurt from skim milk + 14% PWP5 + microencapsulated fish oil, 2; yogurt from skim milk + 12% PWP4 + fish oil, 3; yogurt from skim milk + 12% WPC+ fish oil, 4; yogurt from full-fat milk, 5; yogurt from semi-skimmed milk, 6; and yogurt from 75% skimmed milk, 7. Values with different superscript letters indicates significant difference ($P<0.05$)relative to type 5 (control).

cant difference (p>0.05) between the two different addition levels. It is known that yogurt viscosity varies depending on the type of milk protein and is positively related with higher total solids and protein content [Jumah *et al.*, 2001; Wang *et al.*, 2012]. Heat treatment can increase molecular weight for whey protein, which has a more effective hydrodynamic volume ratio than globular protein and leads to an increase in viscosity [Wang *et al.*, 2013; Nguyen *et al.*, 2015]. Another approach to improve the viscosity and gel strength of yogurt is to further increase the total solids level of the yogurt. The higher the PWP contents, the higher the viscosity of yogurts [Shaker *et al.*, 2000]. In Figure 6 B, the low-fat yogurt samples (types 6 and 7) showed lower apparent viscosity (p<0.01), but the low-fat yogurt incorporated with PWP (types 1, 2 and 3) showed no significant difference compared with full-fat yogurt.

Lucey [2004] who added WPC (without any treatment) to milk, which resulted in a reduction in the viscosity, showed that native whey proteins do not contribute to the gel matrix. It is thought that native whey proteins may act as a structure breaker in acid milk gels and do not interact with casein particles during the acidification. However, after the heat treatment, PWP may become an important cross-linking agent through the heat-induced exposure of previously buried hydrophobic groups. This may be responsible for the increase of the viscosity in yogurts with PWP.

Sensory analysis

The most common sensory attributes relating to yogurt texture are viscosity and smoothness. Results of the sensory evaluation of the yogurt samples on a scale from 1 (very bad) to 10 (excellent) are shown in Table 5. In general, yogurts of types 1 and 2 (microencapsulated fish oil in PWP) showed no significant difference from yogurt type 5 (full-fat yogurt) except for fishy smell caused by fish oil (P<0.05). Yogurt type 3 was scored lower than yogurts of type 1 and 2 but higher than yogurt type 4 on whey separation, lipid separation, fishy smell, and acceptability for the reason that PWP has a good binding ability with fat-soluble substances which avoid lipids separation on the surface of yogurt and reduce releasing fishy smell. Yogurts of type 6 and 7 (low-fat yogurt) showed more whey separation and worse firmness, palatability, and viscosity than full-fat yogurt and low-fat yogurt with varying PWP levels. It indicated that PWP could play the role of fat in yogurt holding aqueous phase, and reduce the whey separation, which was in accordance with previous research [Li & Guo, 2006]. Yogurts of types 1 and 2 tasted as good as full-fat yogurt, and had no significant difference on acceptability, indicating PWP4 and PWP5 were acceptable.

CONCLUSIONS

Almost 85% of the particle size distribution of polymerized whey protein (PWP) was in the range of 1106±158 nm, and the PWP was thermally stable between 50 and 90°C. Addition of PWP resulted in the low-fat yogurts textural characteristics resemble to those of full-fat yogurt. Yogurts incorporated with PWP presented higher firmness, springiness and adhesiveness, but lower cohesiveness than these of any other low-fat yogurts. Use of PWP to encapsulated fish oil could effectively mask the fishy flavor in the yogurts.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Abhyankar A.R., Mulvihill D.M., Auty M.A.E., Combined confocal microscopy and large deformation analysis of emulsion filled gels and stirred acid milk gels. Food Struct., 2014, 127–136.
2. Akalin A.S., Unal G., Dinkci N., Hayaloglu A.A., Microstructural, textural, and sensory characteristics of probiotic yogurts fortified with sodium calcium caseinate or whey protein concentrate. J. Dairy Sci., 2012, 95, 3617–3628.
3. Alting A.C., van de Velde F., Kanning M.W., Burgering M., Mulleners L., Sein A., Buwalda P., Improved creaminess of low-fat yoghurt: The impact of amylomaltase-treated starch domains. Food Hydrocolloid., 2009, 23, 980–987.
4. Andoyo R., Guyomarc'h F., Cauty C., Famelart M.H., Model mixtures evidence the respective roles of whey protein particles and casein micelles during acid gelation. Food Hydrocolloid., 2014, 37, 203–212.
5. Bernard C., Regnault S., Gendreau S., Charbonneau S., Relkin P., Enhancement of emulsifying properties of whey proteins by controlling spray-drying parameters. Food Hydrocolloid., 2011, 25, 758–763.
6. Bertolami M.C., Faludi A.A., Batlouni M., Evaluation of the effects of a new fermented milk product (Gaio) on primary hypercholesterolemia. Eur. J. Clin. Nutr., 1999, 53, 97–101.
7. Boye J.I., Alli I., Thermal denaturation of mixtures of alpha-lactalbumin and beta–lactoglobulin: a differential scanning calorimetric study. Food Res. Int., 2000, 33, 673–682.
8. Brauss M.S., Linforth R.S.T., Cayeux I., Harvey B., Taylor A.J., Altering the fat content affects flavor release in a model yogurt system. J. Agr. Food Chem., 1999, 47, 2055–2059.
9. Brennan J.G., Food texture and viscosity: Concept and measurement by M.C. Bourne, Academic Press, New York. Food Chem., 1984, 13, 80–81.
10. Damin M.R., Minowa E., Alcantara M.R., Oliveira M.N., Effect of cold storage on culture viability and some rheological properties of fermented milk prepared with yogurt and probiotic bacteria. J. Texture Stud., 2008, 39, 40–55.
11. Ding J., Wang C.L., Chen M.H., Wang Y.R., Li F.J., Li Y.D., Gu Q.M., Qiao Q., Wu J.P., Effect of fat replacers from whey protein on the quality of yoghourt. Modern Food Sci. Tech., 2011, 27, 766–767.
12. Dissanayake M., Ramchandran L., Piyadasa C., Vasiljevic T., Influence of heat and pH on structure and conformation of whey proteins. Int. Dairy J., 2013, 28, 56–61.
13. Estrada J.D., Boeneke C., Bechtel P., Sathivel S., Developing a strawberry yogurt fortified with marine fish oil. J. Dairy Sci., 2011, 94, 5760–5769.

14. Fitzsimons S.M., Mulvihill D.M., Morris E.R., Denaturation and aggregation processes in thermal gelation of whey proteins resolved by differential scanning calorimetry. Food Hydrocolloid., 2007, 21, 638–644.
15. Floris R., Bodna´r I., Weinbreck F., Alting A.C., Dynamic rearrangement of disulfide bridges influences solubility of whey protein coatings. Int. Dairy J., 2008, 18, 566–573.
16. Guilmineau F., Kulozik U., Influence of a thermal treatment on the functionality of hen's egg yolk in mayonnaise. J. Food Eng., 2007, 78, 648–654.
17. Guven M., Yasar K., Karaca O.B., Hayaloglu A.A., The effect of inulin as a fat replacer on the quality of set-type low-fat yogurt manufacture. Int. J. Dairy Tech., 2005, 58, 180–184.
18. Herrero A.M., Requena T., The effect of supplementing goats milk with whey protein concentrate on textural properties of set-type yogurt. Int. J. Food Sci. Tech., 2006, 41, 87–92.
19. Janhoj T., Ipsen R., Effect of pre-heat treatment on the functionality of microparticulated whey protein in acid milk gels. Milchwissenschaft-Milk Sci. Int., 2006, 61, 131–134.
20. Jumah R.Y., Shaker R.R., Abu-Jdayil B., Effect of milk source on the rheological properties of yogurt during the gelation process. Int. J. Dairy Tech., 2001, 54, 89–93.
21. Kang J., Guo Q., Phillips G.O., Cui S.W., Understanding the structure-emulsification relationship of gum ghatti – A review of recent advances. Food Hydrocolloid., 2014, 42, 187–195.
22. Komatsu T.R., Buriti F.C.A., da Silva R.C., Lobo A.R. C.C., Gioielli L.A., Saad S.M.I., Nutrition claims for functional guava mousses produced with milk fat substitution by inulin and/or whey protein concentrate based on heterogeneous food legislations. LWT – Food Sci. Tech., 2013, 50, 755–765.
23. Lam R. S. H., Nickerson M. T., The effect of pH and temperature pre-treatments on the structure, surface characteristics and emulsifying properties of alpha-lactalbumin. Food Chem., 2015a, 173, 163–170.
24. Lam R. S. H., Nickerson M. T., The effect of pH and temperature pre-treatments on the physicochemical and emulsifying properties of whey protein isolate. LWT-Food Sci. Technol., 2015b, 60, 427–434.
25. Li J.C., Guo M.R., Effects of polymerized whey proteins on consistency and water-holding properties of goat's milk yogurt. J. Food Sci., 2006, 71, 34–38.
26. Loch J., Polit A., Gorecki A., Bonare k. P., Kurpiewska K., Dziedzicka-Wasylewska M., Lewinski K., Two modes of fatty acid binding to bovine β-lactogobulin – crystallographic and spectroscopic studies. J. Mol. Recognit., 2011, 24, 341–9.
27. Lucey J.A., Cultured dairy products: an overview of their gelation and texture properties. Int. J. Dairy Tech., 2004, 57, 77–84.
28. Manoi K., Rizvi S.H., Physicochemical changes in whey protein concentrate texturized by reactive supercritical fluid extrusion. J. Food Eng., 2009, 95, 627–635.
29. McCowen K.C., Bistrian B.R., Essential fatty acids and their derivatives. Curr. Opin. Gastroen., 2005, 21, 207–215.
30. McCowen K.C., Ling P.R., Decker E., Djordjevic D., Roberts R.F., Coupland J.N., Bistrian, B.R., A simple method of supplementation of omega-3 polyunsaturated fatty acids: Use of fortified yogurt in healthy volunteers. Nutr. Clin. Prac., 2010, 25, 641–645.
31. Milo-Ohr L., Nutraceuticals and functional foods. Food Tech., 2002, 56, 67–70.
32. Mitidieri F.E., Wagner J.R., Coalescence of o/w emulsions stabilized by whey and isolate soybean proteins. Influence of thermal denaturation, salt addition and competitive interfacial adsorption. Food Res. Int., 2002, 35, 547–557.
33. Modler H.W., Kalab M., Microstructure of yogurt stabilized with milk proteins. J. Dairy Sci., 1983, 66, 430–437.
34. Nguyen H.T.H., Ong L., Kentish S.E., Gras S.L., Homogenisation improves the microstructure, syneresis and rheological properties of buffalo yoghurt. Int. Dairy J., 2015, 46, 78–87.
35. Nishinari K., Kohyama K., Kumagai H., Funami T., Bourne M.C., Parameters of Texture Profile Analysis. Food Sci. Technol. Res., 2013, 19, 519–521.
36. Oliveira M.N., Sodini I., Remeuf F., Corrieu G., Effect of milk supplementation and culture composition on acidification, textural properties and microbiological stability of fermented milks containing probiotic bacteria. Int. Dairy J., 2001, 11, 935–942.
37. Paseephol T., Small D.M., Sherkat F., Rheology and texture of set yogurt as affected by inulin addition. J. Texture Stud., 2008, 39, 617–634.
38. Pearce K.N., Kinsella J.E., Emulsifying properties of proteins: evaluation of a turbidimetric technique. J. Agr. Food Chem., 1978, 26, 716–723.
39. Perez A.A., Andermatten R. B., Rubiolo A. C., Santiago L.G., β-Lactoglobulin heat-induced aggregates as carriers of polyunsaturated fatty acids. Food Chem., 2014, 158, 66–72.
40. Purwanti N., Smiddy M., van der Goot A. J., de Vries R., Alting A., Boom R., Modulation of rheological properties by heat-induced aggregation of whey protein solution. Food Hydrocolloid., 2011, 25, 1482–1489.
41. Puyol P., Perez M., Horne D.S., Heat induced gelation of whey protein isolate (WPI): effect of NaCl and protein concentration. Food Hydrocolloid., 2001, 15, 233–237.
42. Ryan K.N., Vardhanabhuti B., Jaramillo D.P., van Zanten J.H., Coupland J.N., Foegeding E.A., Stability and mechanism of whey protein soluble aggregates thermally treated with salts. Food Hydrocolloid., 2012, 27,411–420.
43. Sandoval-Castilla O., Lobato-Calleros C., Aguirre-Mandujano E., Vernon-Carter E.J., Microstructure and texture of yogurt as influenced by fat replacers. Int. Dairy J., 2004, 14, 151–159.
44. Segat A., Misra N.N., Fabbro A., Buchini F., Lippe G., Cullen P.J., Innocente N., Effects of ozone processing on chemical, structural and functional properties of whey protein isolate. Food Res. Int., 2014, 66, 365–372.
45. Shah N.P., Functional food from probiotics and prebiotics. J. Food Tech., 2001, 55, 46–53.
46. Shaker R.R., Jumah R.Y., Abu-Jdayil B., Rheological properties of plain yogurt during coagulation process: impact of fat content and preheat treatment of milk. J. Food Eng, 2000, 44, 175–180.
47. Sloan A.E., Top10 trends to watch and work on third biannual report. J. Food Tech., 2001, 55, 38–40, 42, 44–50, 52, 54, 56, 58.
48. Sodini I., Remeuf F., Haddad S., Corrieu G., The relative effect of milk base, starter, and process on yogurt texture: a review. Crit. Rev. Food Sci., 2004, 44, 113–137.
49. Srisuvor N., Chinprahast N., Prakitchaiwattana C., Subhimaros S., Effects of inulin and polydextrose on physicochemical and sensory properties of low-fat set yoghurt with probiotic-cultured banana purée. LWT – Food Sci. Technol., 2013, 51, 30–36.
50. Sullivan S.T., Khan S.A., Eissa A. S., Whey protein: functionality and foaming under acidic conditions. 2008, *in:* Whey processing,

functionality and health benefits ames.(eds. C.I. Onwulata, P.J. Huth). Wiley-Blackwell, IW, USA, pp. 99–132.

51. Torres I.C., Janhøj T., Mikkelsen B., Østergaard, Ipsen R., Effect of microparticulated whey protein with varying content of denatured protein on the rheological and sensory characteristics of low-fat yoghurt. Int. Dairy J., 2011, 21, 645–655.
52. Varunsatian S., Watanabe K., Hayakawa S., Nakamura R., Effects of Ca^{2+}, Mg^{2+} and Na^{+} on heat aggregation of whey protein concentrates. J. Food Sci., 1983, 48, 42–70.
53. Verheul M., Roefs S.P.F.M., de Kruif C.G., Heat induced denaturation and aggregation of β-lactoglobulin: influence of sodium chloride. 1995, *in:* Food Macromolecules and Colloids (eds. E. Dickenson, D. Lorient). UK, Royal Society of Chemistry, Cambridge, pp. 437–444.
54. Wang G.R., Zhang T.H., Ahmad S., Cheng J.J., Guo M.R., Physicochemical and adhesive properties, microstructure and storage stability of whey protein-based paper glue. Int. J. Adhes. Adhes., 2013, 41, 198–205.
55. Wang W.B., Bao Y.H., Hendricks G.M., Guo M.R., Consistency, microstructure and probiotic survivability of goats' milk yogurt using polymerized whey protein as a co-thickening agent. Int. Dairy J., 2012, 24, 113–119.
56. Wijayanti H.B., Bansal N., Deeth H.C., Stability of whey proteins during thermal processing: A Review. Compr. Rev. Food Sci. F., 2014, 13, 1235–1251.
57. Xie Y.L., Zhou H.M., Zhang Z.R., Effect of relative humidity on retention and stability of vitamin A microencapsulated by spray drying. J. Food Biochem., 2007, 31, 68–80.
58. Yan X.D., Wang C.Y., Yun Z.Y., Qin W.L., Development of yoghurt with high protein and zero fat. J. China Dairy Ind., 2012, 40, 2, 57–59.
59. Zhai J.L., Hoffmann S.V., Day L., Lee T.H., Augustin M.A., Aguilar M.I., Wooster T.J., Conformational changes of alpha-lactalbumin adsorbed at oil–water interfaces: Interplay between protein structure and emulsion stability. Langmuir, 2012, 28, 2357–2367.
60. Zhang T., McCarthy J., Wang G., Liu Y., Guo M., Physiochemical properties, microstructure, and probiotic survivability of low--fat goats' milk yogurt using heat-treated whey protein concentrate as fat replacer. J. Food Sci., 2015, 80, M788-M794.
61. Zhang T.H., Jiang N., Guo M.R., Functional properties of polymerized whey protein after encapsulation of oils. J. Food Sci. Tech., 2013, 31, 21–25(in Chinese; English abstract).

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