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

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Key words: polymerized whey protein, fat replacer, textural characteristics, sensory evaluation

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. Yoghurts 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

Yoghurt 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 yoghurt 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 amyloglucosidase-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 [McCowan & Bistrian, 2005]. McCowan 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 oxidation 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 Cooperative Group, New Zealand) was reconstituted at a pro-
TABLE 1. Formulations of polymerized whey protein with and without fish oil.

<table>
<thead>
<tr>
<th></th>
<th>WPC 80 (g/100 g)</th>
<th>DHA+EPA (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWP0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>PWP1</td>
<td>10</td>
<td>2200</td>
</tr>
<tr>
<td>PWP2</td>
<td>10</td>
<td>1900</td>
</tr>
<tr>
<td>PWP3</td>
<td>10</td>
<td>1600</td>
</tr>
<tr>
<td>PWP4</td>
<td>10</td>
<td>1300</td>
</tr>
<tr>
<td>PWP5</td>
<td>10</td>
<td>1000</td>
</tr>
</tbody>
</table>

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).

dition 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 physicochemical 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.

<table>
<thead>
<tr>
<th>Yogurt types</th>
<th>Skimmed milk/g</th>
<th>FR types</th>
<th>FR ratio/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>140.7</td>
<td>PWP1</td>
<td>6</td>
</tr>
<tr>
<td>B</td>
<td>137.7</td>
<td>PWP2</td>
<td>8</td>
</tr>
<tr>
<td>C</td>
<td>134.7</td>
<td>PWP3</td>
<td>10</td>
</tr>
<tr>
<td>D</td>
<td>131.7</td>
<td>PWP4</td>
<td>12</td>
</tr>
<tr>
<td>E</td>
<td>128.7</td>
<td>PWP5</td>
<td>14</td>
</tr>
</tbody>
</table>

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).

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:

\[
EAI \left( \text{m}^{-2} \text{g}^{-1} \right) = \frac{2 \times 2.303 \times A \times D}{L \times O \times C \times 10^{-4}}
\]

where A is the absorbance at 500 nm, L is the path length of the cuvette (1 cm), D is the dilution factor, O is the volumetric fraction of oil, C is the mass of protein per unit volume of aqueous phase before the emulsions 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 J g^{-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 constant 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-DYS 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.

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 3. The formulations of different types of yogurts.

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

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 30, 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 m²g⁻¹ (PWP modified without homogenization, and was held for 20 min at 85°C) to the maximum 150.5 m²g⁻¹ (PWP modified after homogenization in 20 MPa, and was held for 30 min 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].
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

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

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

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**TABLE 4. Average particle size of polymerized whey protein.**

<p>| Record |</p>
<table>
<thead>
<tr>
<th>T/°C</th>
<th>Z-Ave / d.nm</th>
<th>PdI</th>
<th>Mean Count Rate / kcps</th>
<th>Derived Count Rate / kcps</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.00</td>
<td>266.60</td>
<td>0.50</td>
<td>197.30</td>
<td>139286.60</td>
</tr>
<tr>
<td>2</td>
<td>25.00</td>
<td>265.30</td>
<td>0.48</td>
<td>197.70</td>
<td>139527.20</td>
</tr>
<tr>
<td>3</td>
<td>25.00</td>
<td>263.30</td>
<td>0.48</td>
<td>196.40</td>
<td>138613.60</td>
</tr>
<tr>
<td>Mean</td>
<td>25.00</td>
<td>265.10</td>
<td>0.49</td>
<td>197.10</td>
<td>139142.50</td>
</tr>
</tbody>
</table>

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**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% WPC + fish oil, 3; yogurt from skin 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.**
tein micelles [Herrero & Requena, 2006]. 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; Sandova l-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-

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

<table>
<thead>
<tr>
<th>Sensory attributes</th>
<th>Yogurt types</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>whey separation</td>
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<td>7.9±0.5 2</td>
<td>7.5±0.9 3</td>
<td>6.8±1.3 4</td>
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<td>5.3±1.1 6</td>
<td>4.3±0.6 7</td>
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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-skinned 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 6B, 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 aseous 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.

REFERENCES


50. Sullivan S.T., Khan S.A., Eissa A.S., Whey protein: functionality and foaming under acidic conditions. 2008, in: Whey processing,


