Application of whey protein emulsion gel microparticles as fat replacers in low-fat yogurt: Applicability of vegetable oil as the oil phase

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ABSTRACT

Low-fat, healthy yogurt is becoming increasingly favored by consumers. In the present study, whey protein emulsion gel microparticles were used to improve the quality of low-fat yogurt, and the effects of vegetable oil emulsion gel as a fat substitute on the qualities of low-fat yogurt were investigated, expecting to obtain healthier and even more excellent quality low-fat yogurt by applying a new method. First, emulsion gel microparticles were prepared, and then particle size distribution of emulsion gel and water holding capacity (WHC), textural properties, rheological properties, microstructure, storage stability, and sensory evaluation of yogurt were carried out. The results showed that yogurt with emulsion gel had significantly superior qualities than yogurt made with skim milk powder, with better WHC, textural properties, rheological properties, and storage stability. The average particle size of whey protein-vegetable oil emulsion gel microparticles was significantly larger than that of whey protein-milk fat emulsion gel microparticles, and the larger particle size affected the structural stability of yogurt. The WHC of yogurt made with whey protein-vegetable oil emulsion gel microparticles (V-EY) was lower (40.41%) than that of yogurt made with whey protein-milk fat emulsion gel microparticles (M-EY; 42.81%), and the textural results also showed that the hardness, consistency, and viscosity index of V-EY were inferior to these of M-EY, whereas no significant differences were found in the cohesiveness. Interestingly, the microstructure of V-EY was relatively flatter, with more and finer network branching. The whey separation between V-EY and M-EY also did not show significant differences during the 14 d of storage. Compared with yogurt made with whey protein, vegetable oil, and skim milk powder, the structure of V-EY remained relatively stable and had no cracks after 14 d of storage. The sensory evaluation results found that the total score of V-EY (62) was only lower than M-EY (65) and significantly higher than that of yogurt made with skim milk powder. The emulsion gel addition improved the sensory qualities of yogurt. Whey protein emulsion gel microparticles prepared from vegetable oil can be applied to low-fat yogurt to replace fat and improve texture and sensory defects associated with fat reduction. Key words: vegetable oil emulsion gel, fat substitute, yogurt, applicability

INTRODUCTION

Yogurt has been increasingly popular among consumers because of its high nutritional value and health benefits associated with bioactive peptides and lactic acid bacteria (Gu et al., 2020). Fat is closely related to the quality of yogurt, which gives yogurt good texture, aroma, and taste. However, excessive intake of fat can cause many chronic diseases, such as diabetes and its complications, coronary heart disease, and kidney disease (Lamarche, 2021). With the growth of economy, people’s consumption concept is changing to the point where low-fat products, including low-fat dairy products, are becoming more vital. Nevertheless, low-fat yogurt is often less well accepted by consumers than whole-fat yogurt because the decrease of fat content can lead to textural defects, resulting in reduced taste and flavor (Ravyts et al., 2011). In recent years, some scholars have found that novel emulsion technologies are available for application to low-fat foods to compensate for the loss of quality caused by fat reduction or substitution. Milk fat emulsions prepared from sodium caseinate and anhydrous fat improved the texture, microstructure, and color properties of low-fat cheddar cheese (Sharma Khanal et al., 2019).

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Emulsion gel is a semi-solid system between liquid and solid, an emerging emulsion technology where the gel network structure is filled with emulsion droplets and has a certain mechanical strength (Dickinson, 2013; Cui et al., 2019). Due to the particular structure and function of the emulsion gels, they are used in sausages (dos Santos et al., 2020), yogurt (Li et al., 2021), and cheese (Wen et al., 2021). The gel matrix is composed of components with gel properties, mainly including proteins and polysaccharides, and the emulsion gels can be divided into 3 types according to the differences of the gel matrix, namely protein emulsion gels, polysaccharide emulsion gels, and composite emulsion gels (Scholten, 2019). Whey protein has excellent nutritional value, rich in bioactive substances, and great emulsification and gelling properties (de Wit, 1998; Gauthier et al., 2006). Whey protein emulsion gel has been shown to be an ideal material for improving food quality (Yan et al., 2019).

Now, in most studies of whey protein emulsion gel, vegetable oil, containing relatively more UFA, is regularly used as the oil phase. It has contributed to the prevention of obesity, cardiovascular, and cerebrovascular diseases, as well as other adverse chronic diseases (Olas, 2020). Furthermore, liquid vegetable oil structuralization by means of emulsion gel is healthier for humans than the traditional hydrogenated processing (Wang et al., 2016). Although many scholars have conducted various studies on whey protein emulsion gel prepared from vegetable oil, few studies have been reported to investigate their effects on the quality of dairy products. A study used milk fat to prepare whey protein emulsion gel for incorporating into cheese, which improved its quality (Li et al., 2022). However, vegetable oil, with a lower melting point, exhibits a liquid state at room temperature compared with milk fat, which may affect qualities of dairy products to some extent. Additionally, the effect of whey protein emulsion gel produced using vegetable oil on the quality of dairy products compared with whey protein emulsion gel prepared with milk fat requires further exploration.

Accordingly, in the present study, vegetable oil and milk fat were emulsified respectively with heat-denatured whey protein to acquire 2 whey protein emulsion gel microparticles, namely whey protein-vegetable oil emulsion gel microparticles (V-EG) and whey protein-milk fat emulsion gel microparticles (M-EG), and then they were applied to replace fat in low-fat yogurt. Subsequently, the particle size distribution of emulsion gel particles and some indicators of low-fat yogurt made with emulsion gel were measured, including water holding capacity (WHC), texture characteristics, rheological properties, microstructure, storage stability, and sensory evaluation. Whey protein-milk fat emulsion gel microparticles was taken as a control to evaluate the suitability of V-EG as a fat substitute in low-fat yogurt, with the aim of designing a healthier and high-quality low-fat yogurt.

**MATERIALS AND METHODS**

**Materials**

Whey protein isolate (WPI) powder was the product of Hilmar Ingredients (89 g/100 g of protein). Both skim milk powder and whole milk powder were produced by Fonterra Cooperative Group Ltd. Skim milk powder contained 2 g/100 g of fat, 35 g/100 g of protein, and whole-fat milk powder contained 27 g/100 g of fat, 25.5 g/100 g of protein. Anhydrous milk fat was bought from Hualin Food Ltd. (99.9 g/100 g of fat). Vegetable oil was bought from Fulinmen Food Marketing Ltd. (corn oil, 56.3 g/100 g of PUFA, 29.4 g/100 g of MUFA). Yogurt starter Yo-Mix 883 (50 Danisco culture units/100 L) was produced by Chr. Hansen Inc., which was composed of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Sucrose was purchased from Yichang Jiaran Ltd. All other chemicals were analytical pure.

**Preparation of Emulsion Gel**

At room temperature, WPI powder was mixed with distilled water at 4% (wt/wt) concentration, and homogenized in a high-speed disperser (FJ200-SH, Shanghai Specimen Model Ltd.) at 8,000 rpm for 3 min to ensure complete dissolution of WPI powder. The WPI solution was then hydrated in a 55°C-water bath for 1 h, and then heated at 90°C for 20 min to make protein denaturation. To obtain the emulsion gel, the WPI solution was taken out, added with a certain amount of 55°C oil, and homogenized at 13,000 rpm for 5 min. Then, quickly cooled to room temperature, the glucono-delta-lactone was added while stirring (Wang et al., 2014). After the emulsion gel was formed, it was stored at 4°C for further use.

**Preparation of Yogurt and Formulation**

Six batches of yogurt were prepared based on the formulation in Table 1. In 2 experimental formulation groups, V-EG and M-EG were separately added to make the yogurt contain 1.5% fat (wt/wt), and in 2 control formulation groups, vegetable oil and milk fat were separately used to ensure the yogurt contained 1.5% fat (wt/wt), and the protein contents were adjusted with WPI. Also, the protein contents of yogurt were ensured to be the same for all 4 groups. The yo-
gurts with only skim milk powder (SMP) and whole milk powder (WMP) added were the skim control and full fat control, respectively.

According to Table 1, milk powder and sucrose were weighed in a beaker. The prepared emulsion gel and distilled water were then added and fully stirred to get a uniform emulsion. The emulsion was preheated at 60°C for 5 min, homogenized at 8,000 rpm for 3 min, pasteurized at 85°C for 15 min, and then cooled down to 43°C. Yogurt starter was added to the emulsion at 0.04% (wt/vol) and fermented at 42°C until the pH of yogurt was about 4.5. Completely fermented yogurt samples were then chilled at 4°C for 24 h for ripening. Three replicates were performed for each sample.

### Determination of Size Distribution of Emulsion Gel

The particle size distribution measurement method was based on Wen et al. (2021) with slight modifications. The particle size distribution of emulsion gel was measured by a laser particle size analyzer (Bettersize 2600, Bettersize Instruments Ltd.) in wet measurement mode. A small amount of samples were taken out and diluted with distilled water (1:10). The manual mode was selected and the test conditions were as follows: universal, water, refractive indices of samples and water: 1.52 and 1.33 and optical density: 10–15%. After cleaning the system with distilled water to show that the sample can be added, the diluted sample was slowly added dropwise to the spiking port of the laser particle size analyzer. When the samples reached the set shading rate, the addition was stopped immediately and the system was used for determination. Three replicates were performed for each sample.

### Determination of WHC

The determination method of WHC referred to Wu et al. (2021) with modifications. A 50-mL plastic centrifuge tube was used and weighted. The mass of it was recorded as $m_0$. Yogurt samples were taken out after being ripened at 4°C for 24 h, and about 20-g samples were added into the centrifuge tube. The mass of yogurt was $m$. Then, samples were centrifuged at 4,000 rpm and 4°C for 20 min in a high-speed refrigerated centrifuge (H1850R, Xiangyi Instruments Ltd.). The centrifuge tube was inverted for 5 min after discarding the supernatant. The centrifuge tube and sample were weighted, and then the total mass of them was recorded as $m_1$. The WHC of yogurt was calculated using the following formula:

\[
\text{WHC} \% = \frac{m_1 - m_0}{m} \times 100\%.
\]

### Texture Properties Analysis

The hardness, consistency, cohesiveness, and index of viscosity of yogurt samples stored at 4°C for 1 d after ripening were measured by a texture analyzer (TA.XT-plus, Stable Micro Systems) with back extrusion rig according to the method of Jiang et al. (2021) with modifications. The measuring probe model was a plate back extrusion, which was a flat disk-shaped metal plunger with a diameter of 40 mm. The measured conditions were set as follows: pretest speed of 6.0 mm/s, test speed of 2.0 mm/s, posttest speed of 10.0 mm/s, distance was 80% of the yogurt height, trigger force of 5.0 g, and data acquisition rate of 400 pulses per second. All tests were repeated for 3 times.

### Rheological Analysis

According to the method of Bulut et al. (2021) with modifications, a HAAKE MARS 60 dynamic rheometer (Thermo Electron) fitted with a cone-plate sensor system (C35/1 Ti: diameter = 35 mm; cone angle = 1) was used to determine the rheological characteristics of yogurt stored at 4°C for 2 d after ripening. The test temperature was set at 25°C, and an appropriate size

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**Table 1. Composition of yogurt samples**

| Sample | Emulsion gel (g) | WPI (g) | Oil (g) | Water (g) | Milk powder (g) | Water (g) | Sucrose (g) | Fat content of yogurt (g/100 g) | Protein content of yogurt (g/100 g) |
|--------|-----------------|---------|--------|-----------|----------------|-----------|------------|-----------------------------|----------------------------------|
| M-EY   | 22.16           | 0.62    | 6.66   | 14.88     | 42.48         | 400.36    | 35.00      | 1.50                        | 3.08                             |
| MY     | 0               | 0.62    | 6.66   | 0         | 42.48         | 415.24    | 35.00      | 1.50                        | 3.08                             |
| V-EY   | 22.16           | 0.62    | 6.66   | 14.88     | 42.48         | 400.36    | 35.00      | 1.50                        | 3.08                             |
| VY     | 0               | 0.62    | 6.66   | 0         | 42.48         | 415.24    | 35.00      | 1.50                        | 3.08                             |
| SMP-Y  | 0               | 0       | 0      | 0         | 0             | 58.24     | 0          | 0.17                        | 2.97                             |
| WMP-Y  | 0               | 0       | 0      | 0         | 0             | 58.24     | 0          | 3.14                        | 2.97                             |

1M-EY = yogurt made with whey protein-milk fat emulsion gel microparticles; MY = yogurt made with whey protein, milk fat and skim milk powder; V-EY = yogurt made with whey protein-vegetable oil emulsion gel microparticles; VY = yogurt made with whey protein, vegetable oil and skim milk powder; SMP-Y = yogurt made with skim milk powder; WMP-Y = yogurt made with whole milk powder.

2The total weight of all yogurt samples is 500 g.
A yogurt sample was slowly transferred to the plate for determination. After calibrating the instrument to zero, the frequency scan test of the sample was performed (frequency range: 0.1–10 Hz; strain: 0.5%).

**Scanning Electron Microscopy**

A method was referred with some modifications (Huo et al., 2015). The clots were removed about 1 cm below the surface layer of yogurt, fixed with 2.5% (vol/vol) glutaraldehyde solution at 4°C for 4 h, and then were rinsed. The rinsing solution in the previous step was aspirated before each rinse. First, the clots were rinsed with phosphate buffer (pH 7.2) for 3 times, about 15 min each time, and then respectively eluted with gradient ethanol with 30, 50, 70, 90, and 100% (vol/vol), about 15 min each time. Then, to degrease the yogurt samples, they were rinsed with chloroform for 3 times, about 20 min each time, and finally rinsed with 100% ethanol for 3 times, about 15 min each time. The yogurt clots after a series of treatments were placed in a flat plate, sealed, and holed. They were then frozen at −80°C for 12 h, and dried in a vacuum freeze dryer (Boyikang Experimental Instrument Ltd.) for 24 h. Before scanning electron microscopy imaging, the samples were fixed on a scanning electron microscopy copper plate and sputter coated with gold to maintain sufficient conductivity to obtain high-quality images.

**Storage Stability Analysis**

The yogurt before fermentation was poured into a sterilized clean storage bottle, sealed, fermented, and matured at 4°C. Based on the method of Onsekizoglu Bagci and Gunasekaran (2016) with modifications, the storage stability of the yogurt samples was evaluated at d 0, 7, 14, 21, and 28 after ripening, respectively. The changes in appearance and state were visually observed, and the whey separation was described.

**Sensory Evaluation**

This study was reviewed and approved by the Tianjin University of Science and Technology IRB, and informed consent was obtained from each subject prior to their participation in the study.

The sensory evaluation method referred to Jiang et al. (2021), with some modifications. Twelve reviewers with some experience in sensory evaluation were recruited to form the evaluation team. Also, informed consent was given, and relevant training was carried out to meet the requirements. Six randomly numbered yogurt samples were evaluated by the reviewers in turn, covering the following 3 main aspects: appearance, mouthfeel, and smell. After each taste of a sample, mouthwash was required to eliminate the influence between different samples. Definitions of sensory evaluation terms and scoring criteria were established. A 10-point intensity criteria (weak: 0–2, moderate: 3–6, strong: 7–10) was adopted for each term, as detailed in Table 2. The final results were represented through radar charts.

**Statistical Analysis**

One-way ANOVA in IBM SPSS Statistics 24 (IBM Corporation) was used for data analysis and Duncan’s multiple-range test was used to compare difference of significances (P < 0.05). The results were expressed as mean ± standard deviation. Three repeated experiments were performed.
RESULTS AND DISCUSSION

Effects of Different Particle Size Distribution of V-EG and M-EG on Sample Network Structure

The particle size distribution of V-EG and M-EG was shown in Figure 1A. The particle size distribution of V-EG was wide and uneven, whereas the particle size distribution of M-EG was more concentrated and relatively uniform. Figure 1B showed that the average particle size of V-EG was significantly larger ($P < 0.05$) than that of M-EG. The size and distribution of whey protein microgel particle is one of the main factors in its capability to emulate fat globules (Michalski et al., 2002). It has been proposed that whether fat globules serve as structural fillers or structural disruptors in the gel network depends on the size of the fat globules and the gel network, with larger fat globules generating larger gel network voids (Michalski et al., 2002; O’Mahony et al., 2005). The large average particle size of V-EG might corrupt the protein gel network structure in simulated fat globules, to some extent, and further certify the constraints of vegetable oil emulsion gel in reinforcing the texture of low-fat yogurt.

Influence of V-EG and M-EG Addition on Improving the WHC of Low-Fat Yogurt

Water holding capacity means the ability of yogurt to retain its own whey (Balpetek Kılıç et al., 2021), which directly affects the acceptability and shelf life of the product and is a vital physical property of yogurt (Almusallam et al., 2021). Both the texture and the size
and distribution of voids in the microstructure of yogurt affect its WHC (Gilbert and Turgeon, 2021). Figure 2 shows the influence of emulsion gel prepared by adding different oil on WHC of samples. The WHC of the experimental formulation, the control formulation, and yogurt made with skim milk powder (SMP-Y) was significantly lower than yogurt made with whole milk powder (WMP-Y; \( P < 0.05 \)). Total solids content in yogurt has the capacity to prevent or reduce whey separation, and high fat and protein content has been demonstrated to be associated with low whey separation (Flores-Mancha et al., 2021). Compared with yogurt made with whey protein-vegetable oil emulsion gel microparticles (V-EY) and yogurt made with whey protein-milk fat emulsion gel microparticles (M-EY), higher WHC was observed in yogurt made with whey protein, vegetable oil, and skim milk powder (VY), and yogurt made with whey protein, milk fat, and skim milk powder (MY), with direct addition of non-heat-denatured whey protein (\( P < 0.05 \)). Some of the binding sites of whey protein in the emulsion gel system have been bound and occupied during the formation of emulsion gel. In other words, directly added whey protein had more sites in yogurt that enable binding with other proteins, and the natural whey protein behaved as a filler to back up the gel network (Lu et al., 2020), resulting in narrower voids and a denser structure in the yogurt gel network structure, which subsequently improved the WHC of yogurt. The WHC of VY and MY yogurt exhibited no significant difference (\( P > 0.05 \)).

Comparison of the experimental formulation groups revealed that the WHC of V-EY yogurt (40.41%) was weaker than that of M-EY yogurt (42.81%; \( P < 0.05 \)), which may be owing to the fact that cross-linking between the vegetable oil droplets and casein particles is less likely to occur, increasing whey separation, which affected the protein gel structure (Barrantes et al., 1996). In particular, oil droplets with high solid fat content can significantly contribute to the strength of the emulsion gel (Geremias-Andrade et al., 2017), and vegetable oil, due to its low melting point and liquid state at room temperature, shaped a lower strength of the emulsion gel than that formed by milk fat, which to some extent affected the stability of the 3-dimensional network structure of yogurt. Therefore, whey protein-vegetable oil emulsion gel was less effective than whey protein-milk fat emulsion gel in enhancing the WHC of low-fat yogurt.

Influence of V-EG and M-EG Addition on Improving the Textural Properties of Low-Fat Yogurt

Texture is another key parameter in evaluating the quality of yogurt, mainly including hardness, consistency, cohesiveness, and index of viscosity. The results of the textural characterization are provided in Table 3, which shows that the hardness of yogurt in the experimental formulation groups were decreased compared with VY and MY (\( P < 0.05 \)). Hardness is an essential indicator of yogurt texture and is tightly correlated with TS, protein level and type, and their interactions (Oliveira et al., 2001). The decrease of hardness of V-EY and M-EY may be a result of emulsion gel particles filling in the porous structure of protein matrix as fat globules, which provided yogurt a smooth and delicate profile. In addition, the set yogurt gel with unheated whey protein showed a small amount of whey separation from the surface, and when yogurt was broken, the gel was large and hard (Jin et al., 2018). This was consistent with the results of the present study in which VY and MY yogurts presented a harder texture. The hardness of V-EY yogurt (222.43 g) was weaker than that of M-EY (243.51 g), indicating that V-EY somewhat improved the texture and structure of yogurt. Due to the fact that milk fat consists of numerous fatty acids that are mainly of SFA, which can participate in the structure of triacylglycerols with various molecular masses and a wide range of crystallization, milk fat has a high solid fat content and tends to exist as a mixture of liquid and crystallized fat at room temperature (Viriato et al., 2018). This property of milk fat increased the strength of the emulsion gel, and the stable emulsion gel system

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Figure 2. Water holding capacity of yogurt samples. M-EY = yogurt made with whey protein-milk fat emulsion gel microparticles; MY = yogurt made with whey protein, milk fat, and skim milk powder; V-EY = yogurt made with whey protein-vegetable oil emulsion gel microparticles; VY = yogurt made with whey protein, vegetable oil, and skim milk powder; SMP-Y = yogurt made with skim milk powder; WMP-Y = yogurt made with whole milk powder. Different letters (a–e) indicate a significant difference (\( P < 0.05 \)). Error bars represent SD.
was better cross-linked to the yogurt protein matrix, presenting higher hardness. On the contrary, most vegetable oils contain mainly UFA and exist in liquid form at room temperature, resulting in structurally unstable emulsion gel that had weak cross-linking with the yogurt protein matrix, leading to a lower hardness of the yogurt.

The consistency value of V-EY yogurt (2,076.33 g∙s) was lower (P < 0.05) in comparison to M-EY (2,389.95 g∙s). By the previous particle size distribution analysis of emulsion gels, the average particle size of V-EG was known to be larger than that of M-EG. The structure of emulsion gel was closely related to the structure of the gel matrix and the structure of the emulsion droplets (Lin et al., 2020). At the same protein concentration, relatively less WPI was adsorbed on the surface of oil droplets in V-EG with larger average particle size compared with M-EG with smaller average particle size, resulting in insufficient resistance to deformation of the V-EG structure (Sala et al., 2009). This meant the gel structure might be altered when it was incorporated into yogurt, resulting in its inability to simulate fat globules well enough to thicken the yogurt.

The cohesiveness reflects the strength of the intermolecular forces within the yogurt and the index of viscosity indicates the force required to break the sample into a state that can be swallowed. From Table 3, it was proved that the viscosity of V-EY yogurt was lower than that of M-EY. However, the viscosity of yogurt also cannot be too high, which may perform as a bad, thick, and mushy feeling (Tamime et al., 1996).

Thus, the hardness, consistency, and index of viscosity of V-EY were not as good as those of M-EY, but no significant differences were found in their cohesiveness. Moreover, the hardness, cohesiveness, and index of viscosity of V-EY were improved compared with SMP-Y. The results suggested that vegetable oil emulsion gel could partially improve the textural properties of low-fat yogurt.

Table 3. Results of textural properties of yogurt samples (expressed as mean ± SD)

| Sample | Hardness (g) | Consistency (g∙s) | Cohesiveness (g) | Index of viscosity (g∙s) |
|--------|--------------|-------------------|-----------------|------------------------|
| M-EY   | 243.51 ± 3.24 | 2,389.95 ± 44.12 | −162.53 ± 30.34 | −229.02 ± 8.68 |
| MY     | 356.52 ± 16.35 | 2,566.17 ± 19.95 | −163.70 ± 0.88  | −236.71 ± 22.44 |
| V-EY   | 222.43 ± 8.89  | 2,076.33 ± 27.10 | −147.88 ± 10.32 | −196.20 ± 1.19 |
| VY     | 267.93 ± 1.75  | 2,664.55 ± 18.51 | −157.09 ± 3.42  | −205.76 ± 1.49 |
| SMP-Y  | 218.60 ± 6.12  | 2,140.21 ± 41.05 | −133.04 ± 3.52  | −154.48 ± 0.39 |
| WMP-Y  | 297.60 ± 1.57  | 3,123.14 ± 31.89 | −272.50 ± 1.40  | −363.39 ± 4.18 |

Values in the same column with different superscript letters are significantly different (P < 0.05).

Influence of V-EG and M-EG Addition on Improving the Rheological Properties of Low-Fat Yogurt

Energy storage modulus (G′) and loss modulus (G″) are the parameters of elasticity and viscosity of viscoelastic materials, respectively (Lucey et al., 2005). As can be seen from Figure 3, all yogurt samples showed similar rheological behavior, and at the beginning of the shear rate, they all had a greater G′ than G″, demonstrating an elastic dominant behavior and exhibiting a solid-state nature (Solowiej et al., 2015). The G′ and G″ values of SMP-Y were lower than that of WMP-Y, illustrating that fat exerted an effect on the rheological properties of yogurt. Likewise, the G′ and G″ values of V-EY yogurt were inferior to that of M-EY. Although the vegetable oil emulsion gel could simulate fat globules populating the yogurt network structure in the mode of a protein gel wrapped with oil droplets, the structure and functional properties of the emulsion gel were strongly dependent on the gel matrix, the filling emulsion, and the interaction between them. Vegetable oil with fluidity had little interaction with protein, resulting in the structure of emulsion gel being easily disrupted, which left the low-fat yogurt structure not well stabilized. This result agreed with the textural results. Rheological properties reflect the sensory quality of yogurt and relevant to the texture of foods (Skriver et al., 1999).

Effects of V-EG and M-EG on the Microstructure of Low-Fat Yogurt

The microstructure of yogurt can play a role in various physical properties such as WHC, viscosity, hardness, and rheological properties of yogurt (Skriver et al., 1999; Lucey, 2002). The microstructure of the 2 types of yogurt, which formulated by emulsion gel, was visualized by scanning electron microscopy, and the results are shown in Figure 4. Yogurt possesses a 3-di-
dimensional fiber network structure, in which numerous regular voids are formed. Based on Figure 4, it was found that the M-EY network structure had smaller voids and was more evenly distributed, and with the reference of M-EY, the V-EY network structure was relatively flatter and more branched, but the void size and distribution in the network were less homogeneous, which was not conducive to the WHC, consistency, and viscosity of yogurt. The microstructure of yogurt was a straightforward reflection of the gel state of the emulsion. The microstructure of yogurt was impaired by vegetable oil globules that had difficulties in cross-linking with protein gel and by vegetable oil that had no solid state like milk fat has, which interfered with the emulsion gel status.

**Influence of V-EG and M-EG Addition on Improving the Storage Stability of Low-Fat Yogurt**

A well-qualified yogurt requires not only a superb texture and flavor immediately after production, but a certain level of storage stability to be enjoyed by consumers (Bierzuńska et al., 2019). When yogurt starts to ferment, its pH decreases as lactic acid bacteria...
break down lactose to yield lactic acid until the pH drops to 4.5 to 4.7 and casein coagulum appears to gather, forming yogurt. During low temperature storage at 4°C, although the number and activity of lactic acid bacteria decline, they are still fermenting, leading to a further decrease in the pH of yogurt and an increase in acidity (Meng et al., 2013). Higher acidity and continued proliferation of lactic acid bacteria may break the protein structure and cause whey separation (Ceniti et al., 2020). From Figure 5, it could be noted that whey separation increased in all yogurts as storage time proceeded. Yogurt made with skim milk powder had a more severe whey separation phenomenon, showing significant whey separation after 7 d, and the rest of the yogurts exhibited a significant increase in whey separation after 21 d. The low-fat yogurt manufactured with emulsion gel displayed decreased whey separation and greater storage stability than VY and MY did. The heat-denatured whey protein acted as a bridge to accelerate the cross-linking of proteins in yogurt, tightening the 3-dimensional protein network (Lucey et al., 1997). Vegetable oil and skim milk powder had

Figure 4. Microstructure images of yogurt samples prepared by adding different emulsion gel. (a) microstructure of yogurt made with whey protein-milk fat emulsion gel microparticles (M-EY); (b) microstructure of yogurt made with whey protein-vegetable oil emulsion gel microparticles (V-EY).

Figure 5. Stability of yogurt samples at d 0, 7, 14, 21, and 28 of storage. (a) yogurt made with whey protein-milk fat emulsion gel microparticles (M-EY); (b) yogurt made with whey protein, milk fat, and skim milk powder (MY); (c) yogurt made with whey protein-vegetable oil emulsion gel microparticles (V-EY); (d) yogurt made with whey protein, vegetable oil, and skim milk powder (VY); (e) yogurt made with skim milk powder (SMP-Y); (f) yogurt made with whole milk powder (WMP-Y).
a slight amount of whey separation than MY. This was a result of the relatively unstable yogurt network structure at the initial stage after production, owing to the small interaction of vegetable oil droplets with proteins, which failed to play a useful role in supporting the structure. In contrast, milk fat globules can interact with proteins to participate in protein matrix formation (Tamime et al., 1995). As the yogurt structure changed during storage, the yogurt structure with vegetable oil added directly became more unstable. Interestingly, no major differences in whey separation between V-EY and M-EY yogurts after the 14th day of storage were observed. However, a greater variance started to appear after the 21st day of storage, with more whey separated from V-EY than from M-EY. This was the consequence of the larger mobility of the vegetable oil droplets on the gel structure of the emulsion, which progressively altered. However, it was undeniable that the presence

Figure 6. Images of emulsion gel and yogurt samples. A: (a) whey protein-milk fat emulsion gel microparticles (M-EG), (b) whey protein-vegetable oil emulsion gel microparticles (V-EG); B: (a) yogurt made with M-EG (M-EY), (b) yogurt made with whey protein, milk fat, and skim milk powder (MY), (c) yogurt made with V-EG (V-EY), (d) yogurt made with whey protein, vegetable oil, and skim milk powder (VY), (e) yogurt made with skim milk powder (SMP-Y), (f) yogurt made with whole milk powder (WMP-Y).
of V-EG improved the storage stability of yogurt, at least for a certain period of time.

**Effects of V-EG and M-EG on Sensory Qualities of Low-Fat Yogurt**

Images of all yogurts are showed in Figure 6B. As previously mentioned, the SMP-Y exhibited poor network structure and smoothness. The radar plot of sensory evaluation of all yogurts is illustrated in Figure 7, and none of the samples presented off-flavors. Equally, SMP-Y had the lowest rating and WMP-Y had the highest rating. The reduced fat content of SMP-Y adversely affected the quality of yogurt. Contrasted with VY and MY, the rating of yogurt with emulsion gel was correspondingly superior, with a tighter structure, a smoother surface, and no whey separation. The V-EY score exceeded that of VY, indicating that after vegetable oil was incorporated into whey protein emulsion gel, the protein gel envelope could form an effective physical barrier, limiting the migration and exposure of vegetable oil droplets and preventing vegetable oil from being vulnerable to oil oxidation (Zhuang et al., 2021). Additionally, it can be found that yogurt prepared with V-EY has a creamy color and higher scores compared with M-EY, which is more satisfying to consumers. Milk fat contains carotene, vitamin A, and other pigments (Queirós et al., 2016), thus giving a yellow color to M-EG, which may cause a slight yellow color to the prepared yogurt. The images of emulsion gel are shown in Figure 6A. However, vegetable oil was not detrimental to the color of yogurt. This was consistent with previous findings (Xie et al., 2017). Color is a critical quality characteristic of dairy products, and the color of milk can be caused by the irregular reflection of light by casein micelles and fat globules (Milovanovic et al., 2020). With smaller droplets of vegetable oil, the light became more dispersed, so the yogurt appeared whiter. In terms of color, V-EY and VY are more acceptable to consumers than WMP-Y. Whey protein aggregates may alter the aroma characteristics of dairy products even at an exceptionally low concentration, and that whey protein bound aromatic compounds much better than casein and possess some aroma retention capacity (Lesme et al., 2020). The presence of emulsion gel provided a superior aroma and upgraded the sensory quality of the yogurt. Encouragingly, the discrepancies in aroma acceptance between V-EY and M-EY were not significant, which indicated that the vegetable oil emulsion gel did not contribute to unfavorable aroma of low-fat yogurt. The nutritional composition of yogurt with added vegetable oil undergoes alterations after fermentation; for example, lipids and proteins are hydrolyzed to produce a variety of free fatty acids and free AA, respectively, and free fatty acids are precursors to multiple types of flavor substances (Xie et al., 2017). In general, V-EY can earn the love of consumers as well.

**CONCLUSIONS**

This study demonstrated that the interaction of emulsion gel with yogurt proteins enhanced network structure stability, resulting in higher WHC, better texture properties, rheological properties, and storage stability of emulsion gel yogurts. However, the V-EG structure was more easily destroyed than that of M-EG, and thus the yogurt network structure was not better stabilized. Whey protein-milk fat emulsion gel microparticles was superior in improving the WHC, texture properties, and rheological properties of the yogurt than that of V-EG. Notably, V-EY had a relatively high sensory evaluation score, especially for its creamy color, and showed no significant differences in whey separation compared with M-EY during 14 d of storage. Therefore, V-EG as a fat replacer in low-fat yogurt can be a promising strategy, and this will bring some reference value for the application of new technologies for liquid vegetable oil structuralization and the production of better quality, more nutritious, and lower cost yogurt.
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REFERENCES

Almusallam, I. A., I. A. Mohamed Ahmed, E. E. Babiker, F. Y. Al-Juhaimi, A. Saleh, A. A. Qasem, S. Al Maiman, M. A. Osman, K. Ghaffoor, H. A. Haji, and A. S. Al-Shawaler. 2021. Effect of date palm (Phoenix dactylifera L.) spiklets extract on the physicochemical and microbial properties of set-type yogurt during cold storage. Lebensm. Wiss. Technol. 148:111762. https://doi.org/10.1016/j.lwt.2021.111762.

Balpetek Külçü, D., E. B. Koggin, Ö. F. Celik, and E. Turabi Yolacan. 2021. Investigation of physicochemical, microbiological, textural, and sensory properties of set-type yogurt with Mentha pulegium extract. Food Process. Preserv. 45:e15540. https://doi.org/10.1111/jfp.15549.

Barrantes, E., A. Y. Tamine, A. M. Sword, D. D. Muir, and M. Kalab. 1996. The manufacture of set-type natural yogurt containing different oils—2: Rheological properties and microstructure. Int. Dairy J. 6:827–837. https://doi.org/10.1016/0958-6946(96)00010-6.

Bierzunska, P., D. Cais-Sokolinska, and A. Yigit. 2019. Storage stability of texture and sensory properties of yogurt with the addition of polymerized whey proteins. Foods 8:548. https://doi.org/10.3390/foods8110548.

Bulut, M., Y. Tunçtürk, and D. Alwazeer. 2021. Effect of fortification of set-type yogurt with different plant extracts on its physicochemical, rheological, textural and sensory properties during storage. Int. J. Dairy Technol. 74:723–736. https://doi.org/10.1111/1471-0307.12803.

Ceniti, C., F. Froiio, A. Gagliardi, D. Britti, D. Paolino, and N. Costanzo. 2020. Observations on passive microstructure for monitoring the fermentation process in yogurt. Int. Dairy J. 102:104604. https://doi.org/10.1016/j.idairyj.2019.10.04604.

Cui, M., Y. Lu, Y. Gao, and L. Mao. 2019. A review on the preparation and application of food emulsion gels. Food Sci. (Beijing, China) 40:323–329.

de Wit, J. N. 1998. Nutritional and functional characteristics of whey proteins in food products. J. Dairy Sci. 81:597–608. https://doi.org/10.3168/jds.S0022-0291(98)75613-9.

Dickinson, E. 2013. Stabilising emulsion-based colloidal structures with mixed food ingredients. J. Sci. Food Agric. 93:710–721. https://doi.org/10.1002/jsfa.6013.

dos Santos, M., P. E. S. Munekata, M. Pateiro, G. C. Magalhães, A. C. S. Barretto, J. M. Lorenzo, and M. A. R. Follonio. 2020. Pork skin-based emulsion gels as animal fat replacers in hot-dog style sausages. Lebensm. Wiss. Technol. 132:109845. https://doi.org/10.1016/j.lwt.2020.109845.

Flores-Mancha, M. A., A. G. Ruiz-Gutiérrez, A. L. Renteria-Monterrubio, R. Sánchez-Vega, J. Juárez-Moya, E. Santallano-Estrada, and A. Chávez-Martínez. 2021. Stirred yogurt added with beetroot extracts as an antioxidant source: Rheological, sensory, and physicochemical characteristics. J. Food Process. Preserv. 45:e16528. https://doi.org/10.1111/jfp.15562.

Gauthier, S. F., Y. Pouliot, and D. Saint-Sauveur. 2006. Immuno-modulatory peptides obtained by the enzymatic hydrolysis of whey proteins. Int. Dairy J. 16:1315–1323. https://doi.org/10.1016/j.idairyj.2006.06.014.

Geremias-Andrade, I. M., N. P. D. B. G. Souki, I. C. F. Moraes, and S. C. Pinho. 2017. Rheological and mechanical characterization of curcumin-loaded emulsion-filled gels produced with whey protein isolate and xanthan gum. Lebensm. Wiss. Technol. 86:166–173. https://doi.org/10.1016/j.lwt.2017.07.063.
ent fat globule size distributions. J. Dairy Res. 72:338–348. https://doi.org/10.1017/S0022029905001044.

Olas, B. 2020. Biochemistry of blood platelet activation and the beneficial role of plant oils in cardiovascular diseases. Adv. Clin. Chem. 95:219–243. https://doi.org/10.1016/bs.acc.2019.08.006.

Oliveira, M. N., I. Sodini, F. Remeuf, and G. Corrieu. 2001. Effect of milk supplementation and culture composition on acidification, textural properties and microbiological stability of fermented milks containing probiotic bacteria. Int. Dairy J. 11:935–942. https://doi.org/10.1016/S0958-6946(01)00142-X.

Onsekizoglu Bagci, P., and S. Gunasekaran. 2016. Iron-encapsulated cold-set whey protein isolate gel powder—Part 2: Effect of iron fortification on sensory and storage qualities of Yoghurt. Int. J. Dairy Technol. 69:601–608. https://doi.org/10.1111/1471-0307.12316.

Queirós, M. S., R. Grimaldi, and M. L. Gigante. 2016. Addition of olein from milk fat positively affects the firmness of butter. Food Res. Int. 84:69–75. https://doi.org/10.1016/j.foodres.2016.03.017.

Ravyts, F., L. U. C. De Vuyst, and F. Leroy. 2011. The effect of heteropolysaccharide-producing strains of Streptococcus thermophilus on the texture and organoleptic properties of low-fat yoghurt. Int. J. Dairy Technol. 64:536–543. https://doi.org/10.1111/j.1471-0307.2011.00714.x.

Salea, G., T. van Vliet, M. Cohen Stuart, F. van de Velde, and G. A. van Aken. 2009. Deformation and fracture of emulsion-filled gels: Effect of gelling agent concentration and oil droplet size. Food Hydrocoll. 23:1853–1863. https://doi.org/10.1016/j.foodhyd.2009.03.002.

Scholten, E. 2019. Edible oleogels: How suitable are proteins as a structurant? Curr. Opin. Food Sci. 27:36–42. https://doi.org/10.1016/j.cofo.2019.05.001.

Sharma Khanal, B. K., C. Budiman, M. P. Hodson, M. R. R. Plan, S. Prakash, B. Bhandari, and N. Bansal. 2019. Physico-chemical and biochemical properties of low fat Cheddar cheese made from micron to nano sized milk fat emulsions. J. Food Eng. 242:94–105. https://doi.org/10.1016/j.jfoodeng.2018.08.019.

Skriver, A., J. Holstborg, and K. B. Qvist. 1999. Relation between sensory texture analysis and rheological properties of stirred yogurt. J. Dairy Res. 66:609–618. https://doi.org/10.1017/S0022029999003763.

Solowiew, B., P. Glibowsky, S. Muszyński, J. Wydrych, A. Gawron, and T. Jeliński. 2015. The effect of fat replacement by inulin on the physicochemical properties and microstructure of acid casein processed cheese analogues with added whey protein polymers. Food Hydrocoll. 44:1–11. https://doi.org/10.1016/j.foodhydrocol.2014.08.022.

Tamime, A. Y., M. Kalab, D. Muir, and E. Barrantes. 1995. The microstructure of set-style, natural yogurt made by substituting microparticulate whey protein for milk fat. Int. J. Dairy Technol. 48:107–111. https://doi.org/10.1111/j.1471-0307.1995.tb02478.x.

Tamime, A. Y., E. Barrantes, and A. M. Sword. 1996. The effect of starch based fat substitutes on the microstructure of set-style yogurt made from reconstituted skimmed milk powder. Int. J. Dairy Technol. 49:1–10. https://doi.org/10.1111/j.1471-0307.1996.tb02612.x.

Viriato, R. L. S., M. S. Queiros, M. A. S. da Gama, A. P. B. Ribeiro, and M. L. Gigante. 2018. Milk fat as a structuring agent of plastic lipid bases. Food Res. Int. 111:120–129. https://doi.org/10.1016/j.foodres.2018.05.015.

Wang, F. C., A. J. Gravelle, A. I. Blake, and A. G. Marangoni. 2016. Novel trans fat replacement strategies. Curr. Opin. Food Sci. 7:27–34. https://doi.org/10.1016/j.cofo.2015.08.006.

Wang, H., H. Li, Y. Li, Y. Shi, W. Yin, and L. Zhang. 2014. Properties of milk protein gels made by acidification with glucono-δ-lactone. Zhongguo Rupin Gongye 42:24–27.35.

Wen, P., Y. Zhu, J. Luo, P. Wang, B. Liu, Y. Du, Y. Jiao, Y. Hu, C. Chen, F. Ren, C. U. Alejandro, and Y. Li. 2021. Effect of anthocyanin-absorbed whey protein microgels on physicochemical and textural properties of reduced-fat Cheddar cheese. J. Dairy Sci. 104:225–242. https://doi.org/10.3168/jds.2020-18450.

Wu, D., J. Guo, X. Wang, K. Yang, L. Wang, J. Ma, Y. Zhou, and W. Sun. 2021. The direct current magnetic field improved the water retention of low-salt myofibrillar protein gel under low temperature condition. Lebensm. Wiss. Technol. 151:112034. https://doi.org/10.1016/j.lwt.2021.112034.

Xie, B., S. Tang, S. Li, Y. Zheng, X. Hou, and C. Chen. 2017. Effect of different vegetable oils on the qualities, flavors and fermentation characteristics of probiotic yoghurt. Sci. Technol. Food Ind. 38:151–157. https://doi.org/10.13386/j.issn1002-6306.2017.10.021.

Yan, C., D. Fu, D. J. McClements, P. Xu, L. Zou, Y. Zhu, C. Cheng, and W. Liu. 2019. Rheological and microstructural properties of cold-set emulsion gels fabricated from mixed proteins: Whey protein and lactoferrin. Food Res. Int. 119:315–324. https://doi.org/10.1016/j.foodres.2019.02.012.

Zhuang, X., N. Gaudino, S. Clark, and N. C. Acevedo. 2021. Novel lecithin-based oleogels and oleogel emulsions delay lipid oxidation and extend probiotic bacteria survival. Lebensm. Wiss. Technol. 136(P2):110353. https://doi.org/10.1016/j.lwt.2020.110353.

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