Bigels and their application in yogurt

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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<td>BG</td>
<td>Bigel</td>
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<tr>
<td>CFU</td>
<td>Colony Forming Units</td>
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<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
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<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<td>HG</td>
<td>Hydrogel</td>
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<tr>
<td>LHC</td>
<td>Liquid Holding Capacity</td>
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<td>LVR</td>
<td>Linear Viscoelastic Region</td>
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<tr>
<td>MRS</td>
<td>De Man, Rogosa, and Sharpe</td>
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<tr>
<td>OGE</td>
<td>Oleogel Emulsion</td>
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<tr>
<td>PL</td>
<td>Phospholipid</td>
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<tr>
<td>UHT</td>
<td>Ultra-High Temperature</td>
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<td>WHO</td>
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<td>WPC</td>
<td>Whey Protein Concentrate</td>
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ABSTRACT

The probiotic yogurt market is strong not only because of appealing flavor and textural properties, but also because of the potential benefits that probiotics provide to the host. However, probiotic viability can be easily affected by the environment. Edible bigels, recently-developed soft materials, have shown effectiveness in delivering bioactive components to humans. Nevertheless, the application of bigel in food is lacking. The objectives of this study were 1) use bigel technology to improve the survival of probiotics in yogurt and 2) evaluate the physical and rheological properties of yogurt after bigel addition. In this project, bigels were prepared by homogenizing an oleogel (OGE: 16% wt/wt oleogelators (1:1 soy lecithin: stearic acid), 20% wt/wt milk (1:1 sterilized milk: probiotic milk), and 64% wt/wt soybean oil) and a hydrogel (HG: 25% wt/wt whey protein concentrate and deionized water). Lactobacillus acidophilus and Bifidobacterium lactis, suspended in milk, were incorporated into a bigel to evaluate probiotic viability in yogurt during storage. Different levels (10%, 14%, and 18%) of bigels were incorporated into yogurts, with (Swiss-style) and without (sundae-style) agitation, all samples were stored at 4°C. Probiotic viability was monitored via plate counts for six weeks. Rheological and textural properties were conducted. Spontaneous syneresis, liquid holding capacity, as well as the color and oil migration of the samples were measured.

The results showed the total counts of L. acidophilus and B. lactis entrapped in bigels were significantly higher than free bacteria in yogurt after three weeks and five weeks, respectively, which indicated probiotics could be effectively entrapped, and their survival enhanced, in bigel systems. The presence of phospholipids and whey protein in the bigel matrix enhanced probiotic survival. No significant difference in probiotics survival was found between yogurt styles, which indicated that the bigel macrostructure structure might not play a key role in
protecting the probiotic viability in yogurt, but nano- and microstructure likely do. This study also illustrated that the incorporation of bigels into yogurt changed rheological and textural properties of yogurt without bigel, such as increased viscosity, thixotropy, yield stress and firmness, as well as reduced spontaneous syneresis and promoted liquid holding capacity, possibly by interactions of micro- and nano-structures of bigel with proteins in yogurt strengthened the gel structure of yogurt. The Sundae-style yogurt was more susceptible to the liquid loss compare with the yogurt without bigel, suggesting the bigel incorporation method was critical. Bigel technology shows a promising future for potential application in commercial yogurt to improve probiotic viability and yogurt stability.
CHAPTER 1. INTRODUCTION

Thesis Organization

This thesis is comprised of a literature review and two manuscripts. Manuscript authors are members of the Department of Food Science and Human Nutrition at Iowa State University. Dr. Nuria C. Acevedo is the corresponding author for the manuscripts. This thesis concludes with a conclusion and recommended future work.

Literature Review

Yogurt: history and facts

The history of yogurt can be traced back centuries. It was recorded that milk in the Middle East area was carried in a bag made of intestinal gut; the intestinal juices made milk to curdle and sour, thereby preserving the milk from spoilage (Fisberg & Machado, 2015). Around 6000 BC, the health benefits of consuming fermented dairy products was noticed by Indian Ayurvedic (Brothwell et al., 1998). After Stamen Grigorov discovered *Bacillus bulgaricus* (now *Lactobacillus bulgaricus*), Yllia Metchnikoff revealed the association of lactobacilli in yogurt with the longevity of Bulgarians (Fisberg & Machado, 2015). Yogurt was sold as medicine at the beginning of the 20th century because of its health benefits. The first commercial yogurt plant and laboratory were established in 1932 in France; nine years later, the first yogurt plant and laboratory were opened in the United States (Brothwell et al., 1998).

Nowadays, yogurt is categorized as fermented milk but fermented specifically with *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*, according to the *Codex Alimentarius* (WHO/FAO, 2010). It is also required that the final products contain live cultures (WHO/FAO, 2010). This definition has been widely accepted around the world, with some differences from country to country, such as containing bacteria other than starter cultures.
and containing live and active bacteria (Gómez-Gallo et al., 2018). The yogurt regulation in the US is similar to *Codex Alimentarius*, but without mentioning if the cultures in the final product need to be alive or not. Other strains of bacteria, such as *Lactobacillus acidophilus* and *Bifidobacterium lactis*, may be added as well (Requirements for Specific Standardized Milk and Cream, 2017).

Yogurt is an excellent source of several macro- and micronutrients, such as proteins, vitamin D, and calcium (Gómez-Gallo et al., 2018). The fermentation progress of yogurt increases the levels of B vitamins and folates (Gaucheron, 2011), and the acidic environment enhances the bioavailability of some minerals (Moreno Aznar et al., 2013). Moreover, yogurt and yogurt culture have shown promising health benefits such as improving lactose intolerance and constipation (Adolfsson et al., 2004; Parvez et al., 2006). Because of the high nutrition values and potential health benefits, the demand for yogurt has been steadily increasing over the past several years around the world (R.C. Chandan et al., 2017).

The yogurt market is very diverse and competitive. Different styles of yogurt have been developed to satisfy changing consumer preferences. Based on the milk fat content, yogurt can be classified nonfat, low-fat, and full-fat; yogurt can be sold plain, vanilla-flavored, and fruit-flavored (Chandan & O’Reill, 2007). Depending on the manufacturing process, yogurt is classified as Swiss-style, sundae-style, drinkable, whips, and so on (Chandan & O’Reill, 2007). The most common styles are Swiss- and sundae-style yogurt on the market (Lee & Lucey, 2010). Swiss-style yogurt is called stirred or blended yogurt as well, because the fruit preparation is thoroughly blended with fermented yogurt base (Chandan & O’Reill, 2007). For sundae-style, the fermented or unfermented yogurt is covered on the top of fruit preparation. Therefore, another
name of sundae-style is fruit-on-the-bottom. This style of yogurt needs to be blended to mix the fruit preparation before consumption (Chandan & O’Rell, 2007).

Yogurt is a weak gel-like mixture that exhibits a variety of non-Newtonian characteristics, such as time-dependent shear-thinning behavior and viscoelastic properties (Fu et al., 2018; Lee & Lucey, 2010). The dynamic network structure of yogurt is primarily composed of casein micelles and whey protein aggregates that further strengthen the structure (Fu et al., 2018). Once the gel structure experiences rearrangements, the whey held in the yogurt network is expelled from it and then forms a layer on the top of yogurt, which negatively affects the consumer perception of the product (Lee & Lucey, 2010).

**Probiotics and their health benefits**

In 2002, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) released the most widely accepted and adopted definition of probiotics: “live microorganisms which when administrated in adequate amounts confer a health benefit on the host” (FAO/WHO, 2002). Unfortunately, the misuse of the term probiotic and the misleading claims made to consumers became problematic since that definition was published. To reduce these circumstances, an expert panel, including members of the original FAO/WHO Working Group, was organized by the International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2013. A more precise definition of probiotics was provided, which says “live microorganisms that, when administrated in adequate amounts, confer a health benefit on the host” (Hill et al., 2014). Besides survival in the gastrointestinal (GI) tract, a probiotic should be safe, non-pathogenic, and have no antibiotic resistance (Plaza-Diaz et al., 2019). Metabolic byproducts, dead microorganisms, live cultures in fermented food without evidence of health benefit, and undefined fecal microbiota transplants are not considered probiotics (Hill et al., 2014).
Probiotics can provide potential health benefits to different populations, from newborns to the elderly (Plaza-Diaz et al., 2019), including relieving lactose intolerance symptoms (Hertzler & Clancy, 2003; Roškar et al., 2017), maintaining the balance of intestinal flora (Ohland et al., 2013; Shornikova et al., 1997; Szajewska et al., 2001; Walsham et al., 2016), alleviating depression (Akkasheh et al., 2016; Huang et al., 2016; Messaoudi et al., 2011), remitting type 2 diabetes (Ejtahed et al., 2011, 2012; Hariri et al., 2015; Moroti et al., 2012), as well as improving the immune system (Arunachalam et al., 2000; Gill et al., 2001; Lefevre et al., 2015). Generally, probiotics are from the *Lactobacillus* or *Bifidobacterium* genera; other species like *Escherichia coli* Nissle, some species of *Pediococcus* and *Propionibacterium* are also considered probiotics bacteria (Ranadheera et al., 2010).

Thanks to extensive research conducted by different research groups, significant progress has been achieved on probiotics, especially in the field of their mechanism of action. First, probiotics can produce antimicrobial compounds through metabolization that can suppress the growth of pathogens, including nicin and bacitracin (Vandenbergh, 1993), low-molecular-weight substances such as short-chain fatty acids (Oelschlaeger, 2010), as well as deconjugated bile salts, which are derivatives of bile salts, that can effectively inhibit bacteria (Oelschlaeger, 2010). Secondly, probiotics show anti-adhesive effects. Because probiotics can attach to the epithelial cells and co-aggregate, they can form a protective layer to prevent invasion of pathogens (Schachtsiek et al., 2004). In addition, probiotics are able to compete with pathogens for the same binding receptor and nutrients, consequently preventing the colonization of pathogens (Oelschlaeger, 2010). Moreover, the direct attachment of probiotics on epithelial cells and the release of soluble factors might activate signaling cascades, therefore leading to the immunomodulation of the host (Oelschlaeger, 2010). Last but not least, some probiotics strains
can bind toxins to reduce the intestinal absorption of these toxins (Markowiak & Ślizewska, 2017), or secrete compounds to inhibit the toxin expression (Asahara et al., 2004), which assist in the detoxification of the host.

**Applications of probiotics in food**

As mentioned previously, probiotics must be alive and administrated in adequate amounts to achieve potential health benefits (Hill et al., 2014). Nevertheless, probiotics are sensitive to the harsh environment. It was suggested that probiotics should maintain at least $10^6$ to $10^7$ CFU/mL or g in a food at the time of consumption (Ranadheera et al., 2013). To help the survival and colonization of probiotics in human GI tract, the food product is often chosen as a carrier of probiotics. Food could act as a physical barrier to protect probiotics from the harsh environment; moreover, other compounds naturally present in food (e.g., oligosaccharides and short-chain fatty acids) might interact with probiotics, thereby maintain the viability of them (Ranadheera et al., 2010).

Species of genera *Lactobacillus* and *Bifidobacterium* are probiotics most frequently used in food products (Sanders & Marco, 2010). Various parameters, including fat content, sugar content, and pH could influence the survival of probiotics in the food matrix (Ranadheera et al., 2010). Dairy products are the most traditional vehicle for delivering probiotics to humans (Ranadheera et al., 2017). Ranadheera et al. (2010) stated that milk fat possibly acts as a buffering agent to protect probiotics from direct exposure of unfavorable environments. A number of researchers explored the successful incorporation of probiotics in yogurt (Kailasapathy et al., 2008; Prasanna et al., 2013), fermented milk (Maganha et al., 2014), ice cream (Ferraz et al., 2012), and cheese (Kasimoğlu et al., 2004).

In order to meet the demand and diet change of consumers, more and more non-traditional and non-dairy based food matrices are being investigated for their potential to protect
and deliver probiotics, such as soya- or cereal-based beverages, fruit or vegetable juices and meat products (Ranadheera et al., 2017). Nevertheless, the protective efficacy depends on the composition of the food. For example, Charalampopoulos et al. (2002) found the growth of *L. reuteri* in malt medium was 1.58 and 1.66 log (CFU/mL) higher than that in barley and wheat media, respectively. The possible reason might be the higher fermentable sugar and higher free amino nitrogen concentration of malt medium (Charalampopoulos et al., 2002). Yoon et al. (2006) reported *L. casei* in fermented cabbage juice totally died off after two weeks of cold storage, which was attributed to the lack of essential nutrients and presence of inhibitory substances in cabbage.

**Survival of probiotics in yogurt**

Although several non-dairy based food matrices have been studied in recent years, fermented dairy products, especially bovine milk as raw material, are still the primary delivery vehicle for the delivery of probiotic bacteria (Nadelman et al., 2017; Chaminda Senaka Ranadheera et al., 2018). Yogurt is considered a typical delivery vehicle for probiotics (Gómez-Gallego et al., 2018). There is not a general requirement or agreement on a specific number of probiotics that must present in yogurt; however it was reported that the bifidobacteria should maintain $10^6$ CFU/g in yogurt to provide therapeutic effects (Shin et al., 2000). It was recommended that a daily consumption of 100 g of probiotic food should contain at least $10^7$ CFU/g to elicit health benefits (Nagpal et al., 2012; Ross et al., 2002).

Unfortunately, several factors reduce the survival of probiotics in yogurt, including competition with starter cultures, low pH, dissolved oxygen, and temperature (Rybka & Kailasapathy, 1995; Talwalkar & Kailasapathy, 2004; Talwalkar et al., 2004).

Post-acidification, a decrease in pH after fermentation and during storage at refrigerated temperature, is mainly caused by the uncontrolled growth of *L. bulgaricus* in this environment.
(Lourens-Hattingh & Viljoen, 2001). The pH of the over-acidified yogurt can reach 3.6, which is lower than the typical yogurt pH that ranges between 4.2 to 4.6 (Batt & Tortorello, 2014; Tamime & Robinson, 2007). The majority of Bifidobacterium spp. are sensitive to pH values below 4.6 (Lourens-Hattingh & Viljoen, 2001). Although it was reported that L. acidophilus is more resistant than Bifidobacterium spp. under high acidity (Boylston et al., 2004; Lankaputhra et al., 1996), low pH environments could inhibit the metabolism activity and growth of L. acidophilus (Chan & Zhang, 2005). According to Lourens-Hattingh & Viljoen (2001), the high numbers of L. bulgaricus are associated with the low survival of L. acidophilus population in yogurt. The hydrogen peroxide produced during yogurt processing and storage might cause the antagonism of L. bulgaricus towards L. acidophilus (Lourens-Hattingh & Viljoen, 2001).

L. acidophilus and Bifidobacterium spp. are facultative anaerobes and anaerobes, respectively, which means their oxygen-scavenging system is reduced or deficient (Talwalkar & Kailasapathy, 2004). In facultative anaerobes and anaerobes oxygen cannot be completely reduced to water, but to hydrogen peroxide. These bacteria do not have the catalase enzyme to break down hydrogen peroxide. As a consequence, the toxic substances produced during metabolism, such as superoxide anion, hydroxyl radical, and hydrogen peroxide, accumulates in the cell and cause death (Talwalkar & Kailasapathy, 2004). Bifidobacterium spp. is less oxygen resistant than L. acidophilus, because they are strict anaerobes. During yogurt production, oxygen could be easily introduced and diffuse into several mixing steps (Meybodi et al., 2020). In addition, oxygen can permeate through yogurt packages during storage. Miller et al. (2002) reported that the dissolved oxygen in yogurt packaged with high-impact polystyrene (HIPS) increased from 20 ppm to 50 ppm for 42 days of storage.
Because *Lactobacillus* and *Bifidobacterium* spp. are mesophilic bacteria, the optimal temperature for these bacteria is between 37 to 43°C. Standard yogurt fermentation temperature may be as high as 45°C (Chandan & O’Rell, 2007); therefore it is not surprising that this temperature suppresses the growth and survival of probiotics.

Studies have revealed that some probiotic yogurts in the market have deficiencies in their viability of probiotics. Ibrahim & Carr (2006) found that only 44 out of the 58 (76%) yogurt product on a North Carolina store contained viable probiotics. According to Shah et al. (2000), the counts of *L. acidophilus* was less than $10^6$ CFU/g in 75% of the products at the expiration date; in 94% of the products, the count of *Bifidobacterium* spp. was below $10^6$ CFU/g; the count of *L. casei* was less than $10^6$ CFU/g in 50% of the products.

**Strategies to preserve probiotics**

Several studies have been conducted to preserve viability of probiotics. Microencapsulation is one of the most widely used technologies to preserve the viability of probiotics and, in turn, extend yogurt shelf life. Homayouni et al. (2008) encapsulated *B. lactis* (BB-12) and *L. casei* (LC-01) in resistant starch and sodium alginate-calcium microcapsules that were incorporated into ice cream. The authors found that the microcapsule protected probiotics well at low temperature without affecting the flavor of ice cream. Pimentel-González et al. (2009) encapsulated *Lactobacillus rhamnosus* by an emulsification technique and found that the resistance of *L. rhamnosus* to bile salts and gastric acid was significantly enhanced after encapsulation. However, some disadvantages are associated with microencapsulation, including small-scale production limitations (Bruschi, 2015), high cost (Rokka & Rantamäki, 2010), and deactivation and injury of probiotics during the process (Desobry et al., 1997).

Whey protein may enhance the survival of probiotics by improving probiotic proliferation (Krunić et al., 2019). According to Antunes et al. (2005), adding WPC into fat-free
yogurts promoted the growth of *L. acidophilus* by 1.8 log (CFU/g). Similar results were also found by Kailasapathy & Supriadi (1996); the partial replacement of dried skim milk by WPC in yogurt production maintained the count of *L. acidophilus* sufficiently high during 21-day storage at 5°C. Janer et al. (2004) found that the growth of *B. lactis* in milk supplemented with 2% WPC was 1.5 log (CFU/mL) higher than that in supplemented milk, which was partially due to the increased whey protein content. The whey protein-probiotic interaction mechanism is still unknown (Zhang et al., 2020), but a possible explanation could be the presence of amino nitrogen (Dave & Shah, 1998), α-lactoalbumina and β-lactoglobulina (Ibrahim & Bezkorovainy, 1994) in WPC. The sulfur-containing amino acids in WPC may be contributing to lowering the redox potential, therefore promoting probiotic viability (Dave & Shah, 1998).

Phospholipids are amphiphilic polar lipids that contain a glycerol or sphingosine backbone, fatty acids, phosphate group, and a nitrogen compound or sugar. Work conducted by Aro et al. (2013) demonstrated that phospholipids derived from oat successfully protected *Bifidobacterium breve* in a phosphate buffer. Soy lecithin is a source of phospholipids. Improved survival of probiotics was found in the presence of soy lecithin (Donthidi et al., 2010; Hu et al., 2015). One drawback of phospholipids is rapid oxidation in determined media and conditions (Reis & Spickett, 2012). Previous research in our laboratory showed that *L. acidophilus* and *B. lactis* had higher counts in MRS broth or yogurt with supplemented phospholipids in 6-week storage (data not published). However, the yogurt with phospholipids oxidized and exhibited undesirable off-flavors. Oleogelation is a technique in which an organic liquid is immobilized in a three-dimensional network formed by molecules called oleogelators. Oleogels have been shown to reduce lipid oxidation effectively (Marangoni & Garti, 2011; Tian & Acevedo, 2018, 2020; Zhuang et al., 2020, submitted). Previous work illustrated that the presence of soy lecithin
in the system provided a protective effect on probiotic bacteria survival while inhibiting lipid oxidation (Zhuang et al., 2020, submitted). When compared with the stearic acid oleogel (no soy lecithin), counts of *L. acidophilus* in soy lecithin-stearic acid oleogel emulsions were higher by 3.61 log (CFU/g) at day 14; counts of *B. lactis* were higher by 4.34 log (CFU/g) at day 35.

**Properties of bigels and their application**

Bigels are novel solid-like soft materials produced by combining a hydrogel and an organogel/oleogel at a high shear rate (Shakeel et al., 2018). Bigels not only maintain the key features and advantages of two gel phases, but also further improve the stability and compatibility of them because both phases are structured (Bollom et al., 2020; Mao et al., 2019; Singh, Ramesh, et al., 2014). Because of that, the bigel structure is complicated, and has been shown to have synergistic improvement of the rheology properties when compared to those of the individual phases (Bollom et al., 2020; Patel et al., 2015). The properties and proportions of the hydrogel and the organogel strongly affect the properties of bigels (Mao et al., 2019; Patel et al., 2015). Bigels have shown advantages as drug delivery systems in pharmaceutical and cosmetical applications because of the presence of both polar and non-polar phases (Mao et al., 2019; Shakeel et al., 2018). For instance, several researchers in recent years have found that bigels successfully delivered lipophilic bioactive components (Behera et al., 2015; Lupi et al., 2016; Singh, Banerjee, et al., 2014), as well as hydrophilic drugs (Ibrahim et al., 2013).

Different bigel matrices have been developed with food-grade ingredients, such as agar- or gelatin- and stearic acid-based bigels (Wakhet et al., 2015), monoglyceride- and κ-carrageenan-based bigels (Zheng et al., 2020), and sunflower oil- and protein-based bigels (Behera et al., 2015); nevertheless limited work has been performed on the entrapment and delivery of probiotics using these systems. Behera et al. (2014) used span-40 and four different polymers (sodium alginate, CMC, maltodextrin, and starch) based bigels to entrap *Lactobacillus*
*plantarum* 299v, and reported a survival above $10^8$ CFU/g in all four bigels after 60 days storage. Previous work in our group (Bollom et al., 2020, *in press*) demonstrated that soy lecithin-stearic acid and whey protein bigels significantly enhanced the survival of *Lactobacillus acidophilus* and *Bifidobacterium lactis* throughout a gastrointestinal model system. Up to date, no work has been reported on the incorporation of bigels into food products. 

**Overall goal of this research**

Edible bigel are a relatively unexplored novel technology that have promising potentials in delivering bioactive compounds. Bigels have been explored as delivery systems for pharmaceutical and cosmetic applications. Until now, only two studies investigated bigels for the delivery of probiotics; furthermore, no work has been performed with the objective to incorporate bigels in food. This work presents a new approach to enhance the survival of probiotics in an edible bigel matrix incorporated into a yogurt.

The purpose of the first part of this work was to explore the ability of a soy lecithin-stearic acid and whey protein bigel to enhance the viability of *L. acidophilus* and *B. lactis* when added in different styles of yogurt.

The purpose of the second part of this paper is to investigate the effect of the addition of the produced soy lecithin-stearic acid- and whey protein-based bigel matrix in yogurt characteristics. The rheological, textural, and physical properties of yogurt formulated with bigels will be assessed to determine feasibly of bigel application in a yogurt production.
CHAPTER 2. BIGELS AS PROBIOTIC DELIVERY SYSTEMS IN YOGURT

Modified from a manuscript to be submitted to Journal of Dairy Science

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Abstract

The probiotic yogurt market is strong because of the potential benefits that probiotics provide to the host, such as relieving lactose intolerance symptoms, easing diarrhea, and improving the immune system. However, probiotics are sensitive to processing conditions and the high acidity of yogurt can reduce their survival and limit yogurt shelf life. The objective of this study was to use bigel technology, a novel entrapment system, to improve the survival of probiotics when incorporated into yogurt. Bigels are semisolid systems wherein a polar (hydrogel) and a non-polar (oleogel) phase are mixed to create a material with improved properties over the individual phases. In this study, probiotic bigels were prepared by homogenizing an oleogel (OGE: 16% wt/wt oleogelators (1:1 soy lecithin: stearic acid), 20% wt/wt milk (1:1 sterilized milk: probiotic milk), and 64% wt/wt soybean oil) and a hydrogel (HG: 25% wt/wt whey protein concentrate and 75% deionized water), followed by the incorporation of Lactobacillus acidophilus and Bifidobacterium lactis suspended in milk. Yogurt with 18% wt/wt probiotic bigels incorporated, with (Swiss-style) and without (sundae-style) agitation, as well as Swiss-style yogurt with free probiotics (no bigel) were prepared. Probiotic bigel (no yogurt incorporation) was used as control. Probiotic viability at 4°C was monitored via
plate counts for six weeks. The results showed the total counts of *L. acidophilus* and *B. lactis* entrapped in bigels were significantly higher than free bacteria in yogurt after three weeks and five weeks, respectively, which indicated probiotics could be efficiently entrapped and their survival enhanced in bigel systems. The presence of phospholipids and whey protein in bigel matrix enhanced probiotics survival. No significant difference in probiotics survival was found between yogurt styles, which indicated that the bigel structure may not play the key role in protecting the probiotic viability in yogurt. Bigel application shows a promising future to improve the survival of probiotics in commercial yogurt.

**Key words:** *Lactobacillus acidophilus; Bifidobacterium lactis*; post-acidification; whey protein concentrate; soy lecithin

**Introduction**

The probiotic yogurt market draws much attention because of the potential benefits that probiotics provide to the host such as relieving lactose intolerance symptoms (Hertzler and Clancy, 2003; Roškar et al., 2017), maintaining the balance of intestinal flora (Shornikova et al., 1997; Szajewska et al., 2001; Ohland et al., 2013; Walsham et al., 2016), reducing depression (Messaoudi et al., 2011; Akkasheh et al., 2016; Huang et al., 2016), alleviating type 2 diabetes (Ejtahed et al., 2011, 2012; Moroti et al., 2012; Hariri et al., 2015), as well as improving the immune system (Arunachalam et al., 2000; Gill et al., 2001; Lefevre et al., 2015). Probiotics must be alive and administrated in adequate amounts to achieve health benefits (Hill et al., 2014). It has been recommended that the daily consumption of 100 g of probiotic food should contain at least $10^7$ CFU/g to elicit health benefits (Ross et al., 2002; Nagpal et al., 2012). Yogurt is considered a typical delivery vehicle for probiotics. Unfortunately, several factors reduce the survival of the probiotics in yogurt, including competition with starter cultures, low pH, dissolved oxygen and temperature (Rybka and Kailasapathy, 1995; Talwalkar and Kailasapathy,
Microencapsulation is one of the most widely used technologies to preserve the viability of probiotics and, in turn, extend yogurt shelf life. However, some disadvantages are associated with microencapsulation, including small-scale production limitations (Bruschi, 2015), high cost (Rokka and Rantamäki, 2010), and deactivation and injury of probiotics during the process (Desobry et al., 1997).

Bigels are novel solid-like soft materials produced by combining a gel-like hydrogel and a solid-like organogel at a high shear rate (Shakeel et al., 2018). Bigels have shown advantages as drug delivery systems in pharmaceutical and cosmetical applications (Shakeel et al., 2018; Mao et al., 2019). Different bigel matrices have been developed with food-grade ingredients, such as agar- or gelatin- and stearic acid-based bigels (Wakhet et al., 2015), monoglyceride- and κ-carrageenan-based bigels (Zheng et al., 2020), and sunflower oil- and protein-based bigels (Behera et al., 2015); nevertheless limited work has been performed on the entrapment and delivery of probiotics using these systems. Behera et al. (2014) used span-40 and four different polymers (sodium alginate, CMC, maltodextrin, and starch) based bigels to entrap *Lactobacillus plantarum* 299v, and reported survival above 10^8 CFU/g in all four bigels after 60 d storage. Previous work in our group (Bollom et al., 2020, *in press*) demonstrated that the soy lecithin-stearic acid and whey protein bigels significantly enhanced the survival of *Lactobacillus acidophilus* and *Bifidobacterium lactis* throughout a gastrointestinal model system.

The purpose of this work was to explore the ability of a soy lecithin-stearic acid and whey protein bigel to enhance the viability of *L. acidophilus* and *B. lactis* when added in different styles of yogurt. Our findings present a new approach to enhance the survival of probiotics in food products.
Materials

Granular soy lecithin was obtained from Acros Organics (Geel, Belgium), and stearic acid (95% FCC, FG) was purchased from Fisher Scientific (Hampton, NH, USA). Ultra-high temperature processed whole milk (UHT milk) (Parmalat, Lactalis American Group, Inc., Buffalo, NY, USA) was purchased at a local grocery store. Soybean oil was donated by ADM (Des Moines, IA, USA). Whey protein concentrate was provided by Milk Specialties Global (Eden Prairie, MN, USA). Yogurt starter culture (YO-MIX R05), HOWARU Bifido, and HOWARU Dophilus were donated by Danisco (Danisco USA Inc, Madison, WI, USA).

Methods

Yogurt preparation

UHT milk, in the original carton, was preheated to 43°C in a water bath, all the contents in the carton then transferred to sterilized glass jars. Then the milk was inoculated with 0.05 g/L starter culture (S. thermophilus and L. bulgaricus) and incubated at 43°C for approximately 6 h until a pH of 4.3 ± 0.1 was reached. After incubation, the yogurt was gently stirred aseptically and moved to the 4°C refrigerator overnight prior to sample preparation.

Probiotic milk preparation

The probiotic milk was prepared by adding 0.1 g of Lactobacillus acidophilus and 0.1 g of Bifidobacterium lactis into 10 mL of sterilized MRS broth and grown anaerobically at 37°C for 48 h. Then the sample was centrifuged at 10,000 × g for 10 mins. The resulting supernatant was discarded, and 10 mL of sterilized 2% fat milk was added and vortexed. After the probiotics were dispersed, the mixture was transferred to 69 mL of sterilized 2% fat milk. This process was repeated twice.
**Probiotic bigels preparation**

Probiotic bigels were prepared by blending a hydrogel (HG) and an oleogel emulsion (OGE) in 25:75 HG:OGE ratio. The HG phase was composed of 20% wt/wt whey protein concentrate (WPC 80) as hydrogelator and 80% deionized water as continuous phase. WPC 80 was mixed with deionized water and continuously stirred for 2 h. Then the mixture was transferred to a 4°C refrigerator overnight. Afterward, the pH of the mixture was adjusted to 7.5 by using an 1M NaOH solution, and the ionic strength of the mixture was adjusted to 50 mM using NaCl (Fisher Chemical, Federal Way, WA). Afterwards, the mixture was placed into an 85°C water bath for 30 mins.

The OGE was prepared with 16% wt/wt oleogelators (1:1 soy lecithin: stearic acid), 20% wt/wt milk (1:1 sterilized milk: probiotic milk), and 64% wt/wt soybean oil as continuous phase according to (Gaudino et al., 2019; Bollom et al., 2020). The soy lecithin and stearic acid were added into the soybean oil and continuously stirred on the hot plate at 120°C. Once the soy lecithin and stearic acid dissolved, the mixture was transferred to 85°C water bath.

After both HG and OGE reached 85°C, the OGE was poured onto the HG and the appropriate amount of preheated sterilized milk (85°C) was added. The mixture was homogenized at 13,500 rpm for 2 mins by using a preheated UltraTurrax T25 homogenizer (IKA Works Inc., Wilmington, NC, USA). The bigel mixture was placed in 55°C water bath for 1.5 h to cool down. The preheated probiotic milk (55°C) was added into the mixture once the bigel mixture reached 55°C and the system as homogenized at 8,000 rpm for 45 seconds using a preheated homogenizer.

**Sample preparation**

Swiss-style yogurt with probiotic bigel (SWYPB), Sundae-style yogurt with probiotic bigel (SUYPB), yogurt with free probiotics (YPM), yogurt with no probiotics (Y) and probiotic
bigel not incorporated into yogurt (PB) were prepared (Figure 1). Eighteen percent wt/wt probiotic bigel was incorporated in SWYPB and SUYPB. For SWYPB, probiotic bigel was added in yogurt and stirred for 2 mins at 100 rpm using the IKA overhead stirrer (RW 16 Basic, Wilmington, NC, USA), to mimic blending of fruit prep into Swiss-style yogurt. For the SUYPB sample, the 55°C probiotic bigel mixture was poured in centrifuge tubes and allowed to cool to room temperature to gel. Then the yogurt was poured on the probiotic bigel to mimic fruit-on-the-bottom sundae-style yogurt. For YPM sample, probiotic milk was added in yogurt, and the IKA overhead stirrer was used to mix the probiotic milk and yogurt at 100 rpm for 2 mins to mimic the Swiss-style yogurt preparation, but without the bigel. All samples were prepared in triplicate and stored at 4 °C for appropriate amount of time.

Determination of probiotic viability

During six consecutive weeks, serial dilutions were conducted on all treatments, and selective enumeration of *L. acidophilus* and *B. lactis* was carried out using a pour plate technique (Zhuang et al., 2020, submitted). Before plating, one SUYPB sample was mixed thoroughly to mimic the consumer eating habits; the yogurt of another SUYPB sample was washed off using sterilized deionized water to enable evaluation of the effect of the yogurt on the protective ability of the bigel in the sundae-style yogurt (coded PBSU hereafter). *L. acidophilus* was incubated aerobically on MRS agar (Oxoid, Hampshire, England) with 0.3% w/v bile salt and 1% lactose at 37°C for 72 h. *B. lactis* was incubated anaerobically on MRS agar (Oxoid, Hampshire, England) enriched with 0.3% w/v bile salt and 0.05% w/v L-cysteine at 37°C for 72 h. BD GasPak™ EZ Anaerobe Container Systems (Becton, Dickinson and Company, Franklin Lakes, NJ) was used to create an anaerobic condition. Yogurt without probiotics added (Y) was plated at the same time to confirm the inhibitory effect of MRS-bile agar. Each sample was plated in duplicate.
Determination of pH

The pH of sample containing yogurt (Y, YPM, SWYPB and SUYPB) were determined weekly using a pH meter at 4°C. All samples were measured in triplicate.

Statistical analysis

One-way ANOVA and comparison of means by Tukey’s multiple comparisons test were carried out using JMP Pro 13 (SAS, Cary, NC, USA). All p < 0.05 were considered significant differences.

Results and Discussion

The pH changes of the four yogurt samples are presented in Figure 2. The pH of the yogurts with probiotic bigel (SWYPB and SUYPB) were approximately 0.1 larger than that of the yogurt without probiotic bigel samples (Y and YPM) for the entire storage time, but differences were not show significant (p > 0.05). This was probably due to the dilution effect of bigels incorporation. The pH of all yogurt samples decreased approximately 0.3 at the end of the study, suggesting that post-acidification occurred (Figure 2). Post-acidification, a decrease in pH after fermentation and during storage at refrigerated temperature, is mainly caused by uncontrolled growth of L. bulgaricus in this environment (Lourens-Hattingh and Viljoen, 2001). Several studies have shown that post-acidification is related to a decline in probiotic survival in fermented dairy products, including L. acidophilus and Bifidobacterium species (Shah, 2000; Donkor et al., 2006; Kailasapathy et al., 2008). Thus, it was hypothesized that if bigels serve as a protective barrier, probiotic microorganisms may retain viability even in conditions of yogurt post-acidification.

The mean total counts of B. lactis (Table 1) were higher than those of L. acidophilus (Table 2) throughout six weeks of study. This observation is consistent with a previous work reported by (Zhuang et al., 2020, submitted), however it contradicts other works that found L.
Lactobacillus acidophilus to be more resistant than Bifidobacterium spp. despite high acidity (Donkor et al., 2006; Sahadeva et al., 2011). It is possible that the L. acidophilus strain(s) used in the present study might be more sensitive to the high shear rate than B. lactis (Zhuang et al., 2020, submitted). Another possible reason can be the media used for probiotic enumeration in this study. In this study, the recommended level of bile salt (0.15% wt/wt) (IDF, 1995) did not successfully inhibit the growth of starter cultures; therefore 0.3% wt/wt of bile salt was used, which could have reduced the growth of the L. acidophilus. Sahadeva et al. (2011) mentioned that Lactobacilli strains were inhibited more than Bifidobacterium strains in the presence of bile acid.

The counts of L. acidophilus and B. lactis in both SWYPB and SUYPB decreased approximately 1 log (CFU/mL) over 6 weeks storage, which were not significantly different (p > 0.05) when compared with the counts at the beginning of the experiment (Table 1 and Table 2). Demonstrating a positive protective effect of the bigels, a significant decrease of L. acidophilus appeared at week 3 in YPM; the probiotic ultimately died off at week 5 (Table 1). Similarly, at week 3, the count of B. lactis in YPM was significantly lower than that at week 0, but there were still live B. lactis at week 6. These findings supported our hypothesis that, while probiotic viability continuously decrease during yogurt storage and post-acidification, entrapment of probiotic bacteria in bigels can maintain their viability Swiss-style and sundae-style yogurt. Since no differences in counts were observed between both types of yogurt, bigel incorporation method seems not to matter; bigels may be incorporated into either style of yogurt affectively.

An even better way to see the effectiveness of the bigel on protecting the probiotics is to compare the differences in log count (Δlog CFU/g) of SWYPB and YPM (Figure 3A), and SUYPB with YPM (Figure 3B). A positive Δlog value indicates that L. acidophilus or B. lactis
survived better in SWYPB or SUYPB than those when not protected, in YPM. Throughout the 6 weeks of storage time, both SWYPB and SUYPB exhibited positive Δlog (CFU/g) values for \( L. \text{acidophilus} \); furthermore, the differences reached more than 3 logs at week 3, which was maintained until the end of the study. Although the positive Δlog (CFU/g) values for \( B. \text{lactis} \) were not as profound as that of \( L. \text{acidophilus} \) during the first two weeks, the differences continuously increased and ultimately reached more than 3.5 log (CFU/g) at week 6.

The probiotic viability in SWYPB and SUYPB treatments were compared to investigate the effect of the blending the bigel structure into yogurt (Figure 3C). The Δlog (CFU/g) values for these cases were nearly 0 throughout the 6 weeks of storage time for \( L. \text{acidophilus} \) or \( B. \text{lactis} \), with significant differences (Table 1 and 2). This result further confirmed that the mild blending (100 rpm for 2 mins) of the bigel into SWYPB (mimicking Swiss-style fruit prep blending) did not reduce the ability of the bigel structure to protect probiotics during 6 weeks of storage, even with post-acidification. This suggests that the presence of the microscopic and nanoscopic bigel structures played a vital role in the survival of probiotics entrapped in the matrix. This finding contradicts with the previous work of Zhuang et al. (2020, submitted), which reported that a structured stearic acid oleogel did not protect viability of \( L. \text{acidophilus} \) or \( B. \text{lactis} \) when compared with an unstructured canola oil and water system. But the canola oil and water system in that study did not have any structure (macro-, micro- and nanostructure) of the oleogel because it did not contain oleogelators. In contrast, it is possible that the mild agitation (100 rpm for 2 mins) only destroyed the macrostructure of the bigel matrix, but kept the micro- and nano structure; therefore, the probiotics were still entrapped into the intact micro pockets of the bigel. Bollom et al. (2020, in press) reported that bigels effectively protected probiotics during \textit{in vitro} digestion, which was a harsher environment compare with yogurt (i.e., presence
of acid, enzymes, and bile), even though the bigel structure was modified during digestion. Microscopic analysis such as scanning electron microscope analysis could be performed in the future to further investigate the entrapment of probiotics in bigels before and after agitation.

To evaluate the potential negative effect of the yogurt, poured on the surface of the probiotic bigel, on probiotic viability, yogurt was washed off of some samples of SWYPB, and the counts in the bigel layer (PBSU) were compared to counts in the pure probiotic bigel stored without yogurt on top (PB). Figure 3D shows the effect of the yogurt on probiotic viability entrapped in bigels. The Δlog (CFU/g) values were close to 0, suggesting no negative effect of the yogurt on the bigel matrix.

This work has validated the ability of bigels to protect probiotic bacteria. It does not fully explain the mechanism, which is worthy of further research. However, it is likely that the soy lecithin in the oleogel phase, a source of phospholipids, contributed positively. In our previous work, it was found that the presence of soy lecithin in the system provided a protective effect on probiotic bacteria survival (Zhuang et al., 2020, submitted). When compared with the stearic acid oleogel (no soy lecithin), counts of *L. acidophilus* in soy lecithin-stearic acid oleogel emulsions were higher by 3.61 log (CFU/g) at day 14; counts of *B. lactis* were higher by 4.34 log (CFU/g) at day 35. The positive effect of soy lecithin on probiotic viability was also demonstrated by Donthidi et al. (2010); lecithin-encapsulated *Lactobacillus* and *Bifidobacterium* species had a more than 6 log (CFU/mL) survival after 12-week storage at 23°C.

In addition to the PLs contained in the oleogel phase, it is possible that whey protein in the hydrogel phase contributed to the enhanced probiotics survival in the present study. A recent study pointed out that the proliferation of probiotics could be improved by whey protein and whey protein hydrolysate (Krunić et al., 2019). According to Antunes et al. (2005), adding WPC
into fat-free yogurts promoted the growth of *L. acidophilus* by 1.8 log. Similar results were also found in the work of Kailasapathy & Supriadi (1996); the partial replacement of dried skim milk by WPC in yogurt production maintained the count of *L. acidophilus* sufficiently high during 21-day storage at 5°C. Janer et al. (2004) found that the growth of *B. lactis* reached 9.1 log (CFU/mL) in milk supplemented with 2% WPC, which is possibly due to its caseinomacroleptide (CMP) and whey protein content. The count of *B. lactis* improved by 1.5 log cycles after the supplementation of 2% CMP into milk (Janer et al., 2004). The protein-probiotic interaction mechanism is still unknown (Zhang et al., 2020), but a possible explanation could be the presence of amino nitrogen (Dave and Shah, 1998), α-lactoalbumina and β-lactoglobulina (Ibrahim & Bezkorovainy, 1994) in WPC. The sulfur-containing amino acids in WPC may be contributing to lowering the redox potential, therefore promoting probiotic viability (Dave and Shah, 1998).

According to the Code of Federal Regulation Section 131.200 through 131.206, other optional ingredients can be added into the yogurt are dairy based ingredients, sweeteners, flavoring ingredients, color additives and stabilizers. Bigel, as a novel ingredient, does not belong to any of the categories above. At this stage, application of bigels into yogurt needs to be carefully considered in alignment with yogurt standards of identity.

**Conclusion**

This study demonstrated that a soy lecithin-stearic acid and whey protein bigel effectively entrapped and protected *L. acidophilus* and *B. lactis* against post-acidification conditions in yogurt for six weeks. This study demonstrated that the bigel micro- or nanostructure plays an essential role in protecting probiotic viability in yogurt because destruction of macrostructure did not reduce protection effectiveness. Bigel technology may be considered for application in either
Swiss- or Sundae-style yogurt. This approach shows a promising future for bigel applications in commercial yogurt production to improve the survival of probiotics.

**Acknowledgements**

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**Conflict of Interest**

The authors declare no conflict of interest.

**References**


Table 1. Mean total counts (log CFU/g ± SD) of *Lactobacillus acidophilus* in probiotic yogurt without bigels (YPM), Swiss-style yogurt with probiotic bigels (SWYPB) and Sundae-style yogurt with probiotic bigels (SUYPB) (n = 3 replications)

<table>
<thead>
<tr>
<th>Week</th>
<th>YPM log (CFU/g)</th>
<th>SWYPB log (CFU/g)</th>
<th>SUYPB log (CFU/g)</th>
</tr>
</thead>
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<tr>
<td>0</td>
<td>4.18 ± 0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.33 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.27 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1</td>
<td>3.32 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.22 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.23 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>3.52 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.21 ± 0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.09 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>3</td>
<td>0.84 ± 1.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.11 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.01 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>4</td>
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<td>3.30 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a,b</sup>Different superscripts indicate statistical significance (p<0.05), with an * indicating uncountable
Table 2. Mean total counts (log CFU/g ± SD) of *Bifidobacterium lactis* in probiotic yogurt without bigels (YPM), Swiss-style yogurt with probiotic bigels (SWYPB) and Sundae-style yogurt with probiotic bigels (SUYPB) (n = 3 replications)

<table>
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<tr>
<th>Week</th>
<th>YPM log (CFU/g)</th>
<th>SWYPB log (CFU/g)</th>
<th>SUYPB log (CFU/g)</th>
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<td>4.52 ± 0.57&lt;sup&gt;abcd&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a-e</sup>Different superscripts indicate statistical significance (p<0.05)
Figure 2. Changes in pH with storage time of yogurt without probiotics (Y), yogurt with free probiotics (YPM), Swiss-style yogurt with probiotic bigels (SWYPB) and Sundae-style yogurt with probiotic bigels (SUYPB)
Figure 3. Survival difference [$\Delta \log (\text{CFU/g})$] of *Lactobacillus acidophilus* (closed circles) and *Bifidobacterium lactis* (open circles) between (A) Swiss-style yogurt with probiotic bigel (SWYPB) and probiotic yogurt with free probiotics (YPM), (B) Sundae-style yogurt with probiotic bigel (SUYPB) and YPM, (C) SUYPB and SWYPB and (D) probiotic bigel after Sundae-style yogurt was washed off (PBSU) and probiotic bigel not incorporated into yogurt (PB)
CHAPTER 3. PHYSICAL AND RHEOLOGICAL PROPERTIES OF YOGURT INCORPORATED WITH BIGEL

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Abstract

Bigels are unique novel materials produced by shearing the hydrogel and organogel together. Bigels can deliver both lipophilic and hydrophobic drugs or bioactive components to consumers. Our previous study demonstrated that a bigel effectively enhanced the survival of probiotics in yogurt for at least six weeks. This work reports on how the bigel affects yogurt physical properties. In this study, bigels were prepared by homogenizing an oleogel (OGE: 16% wt/wt oleogelators (1:1 soy lecithin: stearic acid), 20% wt/wt milk, and 64% wt/wt soybean oil) and a hydrogel (HG: 25% wt/wt whey protein concentrate and deionized water). Then the bigels were incorporated into yogurt with (Swiss-style) and without (sundae-style) agitation. Three levels of bigel (10%, 14%, and 18%) were blended into yogurt for Swiss-style yogurt. Bigels were added at the bottom of containers at the level of 18% for sundae-style yogurt. Rheological and physical analyses were conducted. Bigel incorporation changed rheological and textual properties of Swiss-style yogurts. Swiss-style yogurt showed higher viscosity, higher anti-shear properties, higher yield stress, and higher firmness. Bigel incorporation decreased degree of spontaneous syneresis and increased the liquid holding capacity of the Swiss-style yogurt as
well. Those changes of rheological and physical properties could be partially explained by the strengthened protein-protein interactions and the addition of another semisolid matrix has different physical and rheological properties. The sundae-style yogurt was more susceptible to the liquid loss compared with the yogurt without bigel, demonstrating that the bigel incorporation method was critical. Bigel application shows a promising future to improve the physical and rheological properties of Swiss-style yogurt.

Key words: whey protein concentrate; soy lecithin; syneresis; texture

Introduction

Yogurt is a weak gel-like mixture that exhibits a variety of non-Newtonian characteristics, such as time-dependent shear-thinning behavior and viscoelastic properties (Fu et al., 2018; Lee & Lucey, 2010). The dynamic network structure of yogurt is primarily composed of casein micelles and whey protein aggregates that further strengthen the structure (Fu et al., 2018). Once the gel structure experiences rearrangements, the whey held in the yogurt network is expelled from it and then forms a layer on the top of yogurt, which negatively affects the consumer perception of the product (Lee & Lucey, 2010).

Bigels are novel solid-like soft materials produced by combining hydrogel and organogel at a high shear rate (Shakeel et al., 2018). Numerous bigels have been developed, such as carbopol- and fish oil-based bigel (Rehman & Zulfakar, 2017), agar- or gelatin- and stearic acid-based bigel (Wakhet et al., 2015), as well as sunflower oil- and protein-based bigel (Beauty Behera et al., 2014). Bigels not only maintain the key features and advantages of two gel phases, but also further improve the stability and compatibility of them because both phases are structured (Bollom et al., 2020; Mao et al., 2019; Singh, Ramesh, et al., 2014). The properties of the hydrogel and the organogel strongly affect the properties of bigels (Mao et al., 2019). Bigels have shown advantages as drug delivery systems in pharmaceutical and cosmetical applications...
because of the presence of both polar and non-polar phases (Mao et al., 2019; Shakeel et al., 2018). For instance, several researchers in recent years have found that bigels successfully delivered lipophilic bioactive components (Behera et al., 2015; Lupi et al., 2016; Singh, Banerjee, et al., 2014), as well as hydrophilic drugs to consumers (Ibrahim et al., 2013). Most of the current research on bigel application was mainly focused on pharmaceutical and cosmetical industry (Shakeel et al., 2018). Although several edible bigels were developed (Bollom et al., 2020; Zheng et al., 2020), no work has been done on the incorporation of bigels into food products.

Our previous work found that bigels effectively protected the probiotic viability in an in vitro digestion model (Bollom et al., in press), as well as in yogurt for six weeks (Zhuang et al., submitted). Therefore, it is important to also characterize how bigels affect the properties of yogurt. The purpose of this paper is to report on findings about the potential application of soy lecithin-stearic acid- and whey protein concentrate-based bigel matrix in yogurt production. Therefore, the objective of this study is to characterize the rheological, textural, and physiochemical properties of yogurt formulated with bigels. Our findings may provide valuable insights into the potential commercialization of bigel incorporated yogurt, and could be useful to researchers attempting to develop more bigel incorporated food products.

**Materials**

Granular soy lecithin was purchased from Acros Organics (Geel, Belgium), and stearic acid (95% FCC, FG) was purchased from Fisher Scientific (Hampton, NH, USA). UHT whole milk (Parmalat, Lactalis American Group, Inc., Buffalo, NY, USA) was purchased at a local grocery store. Soybean oil was donated by ADM (Des Moines, IA, USA). Whey protein concentrate was donated by Milk Specialties Global (Eden Prairie, MN, USA). Yogurt starter
culture (YO-MIX R05) were donated by Danisco (Danisco USA Inc, Madison, WI, USA). Nile Red was obtained from Chem-Impex International (Wood Dale, IL, USA).

Methods

Yogurt preparation

A commercial UHT milk, in the original carton, was preheated to 43°C in a water bath; all the contents in the carton were then transferred to sterilized glass jars. The milk was inoculated with 0.05 g/L starter culture (S. thermophilus and L. bulgaricus) and incubated at 43°C for approximately 6 h until a pH of 4.3 ± 0.1 was reached. After incubation, the yogurt was gently stirred aseptically and moved and stored at 4°C refrigerator overnight prior to sample preparation.

Bigel preparation

Bigels were prepared by blending hydrogel (HG) and oleogel emulsion (OGE) with a ratio of 25:75. The HG phase was composed of 20% w/w whey protein concentrate (WPC80) as hydrogelator and 80% deionized water as a continuous phase. WPC80 was mixed with deionized water and continuously stirred for 2 hours. Then the mixture was transferred to a 4°C refrigerator overnight. Afterward, the pH of the mixture was adjusted to 7.5 by using NaOH solution, and the ionic strength of the mixture was adjusted to 50 mM using NaCl. Protein gelation was induced by heating the mixture at 85°C for 30 mins. The OGE was prepared with 16% w/w oleogelators (1:1 soy lecithin: stearic acid), 20% w/w milk, and 64% w/w soybean oil as the continuous phase according to Gaudino et al. (2019). The soy lecithin and stearic acid were added into the soybean oil and continuously stirred at 120°C. Once the soy lecithin and stearic acid dissolved, the mixture was transferred to an 85°C water bath.

Once both HG and OGE reached 85°C, the OGE was poured onto the HG and half (10% w/w) of the preheated UHT milk (85°C) was added. The mixture was homogenized at 13,500
rpm for 2 mins by using preheated UltraTurrax T25 homogenizer (IKA Works Inc., Wilmington, NC, USA). Then, the bigel mixture was placed in 55 °C water bath for 1.5 h to cool down. Another half (10% w/w) of the preheated UHT milk (55 °C) was added into the mixture once the mixture reached 55 °C and followed by second homogenization at 8,000 rpm for 45 seconds using preheated homogenizer. Then the bigel mixture was cooled down to room temperature to allow gelation.

**Sample preparation**

Swiss-style yogurt with five levels of bigel added: 0%, (Y), 10% (BY10), 14% (BY14) and 18% (BY18) of the total sample weight were prepared by adding appropriate amount of bigel into yogurt and stirred for 2 mins at 100 rpm using IKA overhead stirrer (RW 16 Basic, Wilmington, NC, USA). Sundae-style yogurt (BYSU) was made by pouring 18% w/w of 55°C bigel in the bottom of the container and allowed to cool down to room temperature to gel followed by pouring yogurt on top of the bigel surface. Bigel sample without yogurt (BG) was prepared by pouring 55°C bigel in the container and allowed to gel at the room temperature. All samples were stored at 4 °C.

**Rheological properties**

Rheological properties of the four yogurt samples, BY10, BY14, BY18 and Y, as well as BG were determined under 4 °C on a hybrid rheometer (Discovery HR-2, TA Instruments, New Castle, DE, USA) with a 40 mm steel parallel plate geometry using a 1000 µm gap. Approximately 2 g of sample were removed out of the container using spatula. The sample was allowed to settle between the plates for 5 min to stabilize the structure after each loading. All samples were measured in triplicate.

A thixotropic test was used to characterize the flow behavior of yogurt samples according to the method reported by (Miocinovic et al., 2018) with slight modifications. A flow sweep was
conducted at increasing shear rates (upward flow curve) from 0.01 to 100 s$^{-1}$ and decreasing shear rates (downward flow curve) from 100 to 0.01 s$^{-1}$. Shear stress was recorded automatically, and the hysteresis loop area (Pa s$^{-1}$) was calculated by TA Instruments Trios v5.1.0 software to determine the thixotropic behavior of the sample. The upward curve was fitted to the power law model:

$$\sigma = Ky^n$$

Where $\sigma$ is the shear stress, $K$ is the consistency index, $\gamma$ is the shear rate, and $n$ is the flow behavior index. Apparent viscosity ($\eta_{app}$) at 50 s$^{-1}$ was obtained as well (Marcotte et al., 2001).

An oscillation test was conducted to determine the linear viscoelastic range (LVR) of the samples according to the method described by Miocinovic et al. (2018) with minor modifications. Briefly, strain sweeps were carried out by increasing the strain from 0.001% to 500% at a constant frequency of 1.0 Hz. Shear storage ($G'$) and shear loss ($G''$) moduli were recorded automatically. The end of the LVR was marked by the first point where the average $G'$ value dropped by 10% from the average $G'$ within the LVR, and the corresponding stress at this point is referred to as the yield stress ($\sigma$, Pa) (Acevedo et al., 2012).

**Texture analysis**

Four yogurt sample BY10, BY14, BY18 and Y, were analyzed to determine texture parameters using TA.XT plus texture analyzer (Stable Micro Systems, Surrey, UK). Firmness, cohesiveness, adhesiveness, and springiness values were obtained by conducting texture profile analysis (TPA). Samples were tested in original container without stirring. Two sequential compressions were performed with a 0.5” diameter TA-10 probe. The compression distance was 15 mm, and the trigger force was 2.0 g. The pre-test, test, and post-test speed were 4, 1, 3 mm/s, respectively. All samples were measured in triplicate.
Oil migration

Bigels colored by Nile Red dye were incorporated into the yogurt as described in the sample preparation section. The dye was added before the homogenization of the bigel phase. Oil migration was determined by taking pictures in the same position every week and analyze by visual.

Spontaneous syneresis and liquid holding capacity

Two different styles of yogurt with the same bigel content (BY18, BYSU) were analyzed for spontaneous syneresis and liquid holding capacity. Y and BG were tested at the same time as the control. All samples were measured in triplicate.

The spontaneous syneresis of the yogurt was obtained using the method reported by (Amatayakul et al., 2006). The yogurt container was kept at approximately 45° after being removed from the refrigerator to help in supernatant collection. The supernatant was collected by a needle connected to a syringe and weighed. Spontaneous syneresis was calculated using the equation below:

\[
\text{Syneresis (\%)} = \frac{\text{weight of liquid released}}{\text{total weight of sample}} \times 100
\]

The liquid holding capacity (LHC) of the yogurt sample was determined using the method described by Fu et al. (2018) with modifications. In this study, “liquid” means both free whey and oil. In general, the yogurt sample was centrifuged at 1000 × g for 15 mins at 4°C using a centrifuge, the supernatant was collected and weighed after centrifugation. Sample LHC was calculated using the equation below:

\[
\text{LHC (\%)} = \frac{\text{total weight of sample} - \text{weight of liquid released after centrifugation}}{\text{total weight of sample}} \times 100
\]
**Instrumental color analysis**

Instrumental color analysis of BG, BY10, BY14, BY18 and Y was performed by using a colorimeter (ColorFlex® EZ, HunterLab, Reston, Virginia, USA). L*, a* and b* values were obtained based on CIELab color scale. Chroma (C*) and hue angle (h) were calculated by the following equations:

\[
C^* = (a^{*2} + b^{*2})^{1/2}
\]

\[
h = \arctan(b^*/a^*)
\]

**Statistical analysis**

One-way ANOVA and comparison of means by Tukey’s multiple comparisons test were carried out using JMP Pro 13 (SAS, Cary, NC, USA); p < 0.05 was considered a significant difference between values.

**Results and Discussion**

**Rheological properties**

Viscosity is one of the most critical properties of foods because it affects the mouthfeel and texture of the fluids (Isanga & Zhang, 2009). In our preliminary test, BG was squeezed out of the plate when shear rate was high, the data of BG was not analyzed. All yogurts showed a shear-thinning behavior because decreasing viscosity was found with an increasing shear rate (Figure 5), which can be explained by the shear-induced breakdown of the gel structure and weakening of interactions within the yogurt network (Mohameed et al., 2004). The lactic acid bacteria produce acid during the fermentation, cause aggregation of casein strands, and form a three-dimensional network of yogurt (Tamime & Robinson, 2007). As the shearing rate increases, the casein strands can be broken apart, and the size of the aggregate decreases (Afonso & Maia, 1999). According to Lee & Lucey (2010), the interactions within the yogurt network
include electrostatic and hydrophobic forces, which are considered to be weak physical bonds.

The higher bigel content contributed to the higher viscosity, which might be explained as well as the apparent viscosity at a shear rate of 50 s$^{-1}$, which was reported as an effective oral shear rate (Marcotte et al., 2001). A preliminary study was done by Remeuf et al. (2003), who also reported a higher viscosity in WPC-enriched yogurt.

The breakdown of weak particles in dispersion or the weak interparticle bonds under shear cause the thixotropy (Magenis et al., 2006). Thixotropy is represented by the different area under the upward and downward flow curves (hysteresis loop area), it represents the energy needed to destroy the structure of the material (Miocinovic et al., 2018). According to Figure 4, all yogurt samples exhibited thixotropic behavior after the increasing and decreasing shear rates. The hysteresis loop area of yogurt with bigel was significantly higher than that of the yogurt without bigel, and the loop area increased as the bigel concentration increased (Table 3). This result indicated that yogurt with higher bigel content requires higher energy to break down the gel structure. In addition, the upward flow curves were fitted to power law model, Table 3 showed the resulting parameters. The correlation coefficient for this model were all above 0.92 (data not shown). The consistency indices of all samples are ranged from $1.22 \times 10^{-4}$ to $9.43 \times 10^{-5}$. All yogurt behaved as pseudoplastic fluid ($n < 1$), confirming the non-Newtonian behavior.

Figure 6 displays the amplitude sweep result of yogurt with different bigel contents. Compared with the yogurt without bigel, all samples with bigel incorporation showed significantly higher yield stress, and significant differences were found between different bigel concentrations (Table 3). Higher yield stress indicates the higher stress was required to initiate the flow of the yogurt, and it might be related to the enhanced interactions between protein after bigel addition. These results might relate to the micro- and nano-structure interact with yogurt.
protein, forms a stronger network. Because the yield stress of bigel (62,910 Pa) was much higher than that of yogurt, the increased yield stress could be explained by the addition of a new matrix with different characteristics. Not surprisingly, both storage modulus ($G'$) values and loss modulus ($G''$) values of yogurt with bigel were higher than the yogurt without bigel (Figure 6). Both $G'$ and $G''$ increased as increased bigel concentration.

Overall, the bigel incorporation changed rheological properties of yogurt, such as increased viscosity, thixotropy, as well as yield stress. These is possibly because of the increased interactions of yogurt protein and micro- and nano-structure of bigel, or the addition of another semi-solid matrix. The microstructure of the interphase of bigels and yogurt needs to be elucidated.

**Texture analysis**

The textural properties of yogurt are critical quality criteria. It can be seen from Table 4 that yogurts with bigel had higher firmness, adhesiveness, gumminess, but equal springiness and lower cohesiveness compared with the yogurt without bigel. The increased firmness is consistent with the increased yield stress described in previous section, because Harte et al. (2007) reported that the yield stress highly correlated with the initial firmness of yogurts during sensory assessment. Similarly, the change of texture profile might partially attributed to the addition of other semi-solid matrix with much higher firmness (the firmness of bigel was 151.83 g), or the increased interaction of micro- and nano-structure of bigel with protein in yogurt.

**Oil migration**

The oil migration of the bigel phase in yogurt is shown in Figure 7. The bigel in Swiss-style yogurt was relatively stable; we did not observe any large oil droplets at any concentrations during the storage, which was comparable with the spontaneous syneresis results above.
Nevertheless, the boundary of the bigel particles got blurry during storage. This indicates that oil migration might happen out of bigels during extended storage. For the sundae-style yogurt, the large oil droplets were observed from week 2, and the oil droplet started to accumulate, and continuously rose through the yogurt layer, finally reaching the surface of the yogurt. This indicated that the bigel phase was not stable in sundae-style yogurt, but the reason was still unclear.

**Spontaneous syneresis and liquid holding capacity**

Spontaneous syneresis is a common major visible defect that occurs during refrigeration storage (Bierzuńska et al., 2019). The whey held in the yogurt network is expelled from it and whey accumulates on the top of the yogurt gels, which negatively affects the consumer acceptability of the product (Lee & Lucey, 2010). Both yogurts without bigel and sundae-style yogurt with bigel started showing syneresis at week 3 and continuously increased until the highest degree of syneresis appeared at week 6 (Table 5). It has been reported that lower pH during yogurt storage possibly causes the contraction of the casein network; the reduction of the net negative charge of the casein micelles in yogurt is responsible for the decreasing of electrostatic repulsion and increased casein-casein attraction, therefore causing higher whey protein expulsion (Lee & Lucey, 2010). On the other hand, the bigel sample and Swiss-style yogurt with bigel did not experience any syneresis during the storage (Table 5), which correlates with the oil migration results discussed in previous section. Sundae-style yogurt was significantly more susceptible to the spontaneous syneresis than the Swiss-style yogurt during storage (Table 5). Because both BY18 and BYSU contained the same level of bigel (18%), this difference was very likely related to the different bigel incorporation method. Specifically, the bigel was thoroughly dispersed throughout the swiss-style yogurt, while the bigel was settled on the bottom of the sundae-style yogurt. Thus, compared with the sundae-style yogurt, the bigel in
Swiss-style yogurt has a larger contact area with yogurt, which might increase interactive sites, enhance the protein-protein interactions and form a stronger network to entrap free water. At week 6, the degree of syneresis of sundae-style yogurt was 3.06%, which was significantly lower than that of yogurt without bigel. The possible explanation could be the protein-protein interaction was reinforced near the interphase of bigel and yogurt, or the less syneresis of the bigel.

Except for BG, all yogurt samples showed a significant decrease in liquid holding capacity at the end of the study (Table 6). Different from the results of spontaneous syneresis, the whey separation was observed on the swiss-style yogurt after centrifugation, indicating the bigel yogurt structure was weak, and susceptible to losing water when centrifugal forces were applied. However, a significantly higher LHC of swiss-style yogurt was observed at week 6, suggesting the micro- and nano-structure of bigel promoted the liquid holding capacity during storage. Similarly, more free water could be immobilized within the reinforced network of yogurt. One interesting observation was that the LHC of sundae-style yogurt was significantly lower than those of the other two yogurt samples in every single week, the possible reason might be as the oil droplets arising to the top of the sample, the protein-protein network of yogurt phase was disturbed, led to more water compressed out..

Color analysis

The addition of bigel significantly affected the color of yogurt (Table 7). Yogurt with bigel exhibited significant lower L*, demonstrated that bigel addition led to a less white, darker appearance of yogurt. Because visually the pure bigel was light yellow, it was not surprising that the yogurt with bigel also displayed a significantly yellower color. Although there was a significant increase in redness as bigel concentration increases, the a* values were still below 1, and the changes may not be visually perceived.
Conclusion

This study illustrated that the incorporation of soy lecithin-stearic acid and whey protein bigels into yogurt changed rheological and textural properties of yogurt with reducing spontaneous syneresis and promoted liquid holding capacity, possibly by interactions of micro- and nano-structures of bigel with proteins in yogurt strengthened the gel structure of yogurt. However, the improvement depended on the incorporation method. Swiss-style yogurt may be more suitable for the bigel incorporation. This approach shows a promising future for bigel applications in yogurt production.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

References


Table 3. Rheological properties of yogurt with no bigel (Y), 10% w/w bigel (BY10), 14% w/w bigel (BY14), and 18% w/w bigel (BY18)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield stress (Pa)</th>
<th>Hysteresis loop area (Pa s(^{-1}))</th>
<th>App. viscosity at 50 s(^{-1}) (Pa s)</th>
<th>Consistency index (K, MPa s(^n))</th>
<th>Flow behavior index (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>213.08 ± 15.84(^a)</td>
<td>1850.46 ± 393.44(^a)</td>
<td>0.58 ± 0.03(^a)</td>
<td>9.22 × 10(^{-5})</td>
<td>-0.31</td>
</tr>
<tr>
<td>BY10</td>
<td>402.43 ± 46.51(^b)</td>
<td>2652.74 ± 268.75(^b)</td>
<td>0.85 ± 0.07(^b)</td>
<td>9.43 × 10(^{-5})</td>
<td>-0.31</td>
</tr>
<tr>
<td>BY14</td>
<td>556.70 ± 27.65(^c)</td>
<td>2825.90 ± 235.45(^bc)</td>
<td>0.88 ± 0.05(^bc)</td>
<td>1.22 × 10(^{-4})</td>
<td>-0.26</td>
</tr>
<tr>
<td>BY18</td>
<td>773.68 ± 57.80(^d)</td>
<td>3231.74 ± 130.06(^c)</td>
<td>0.97 ± 0.03(^c)</td>
<td>9.18 × 10(^{-5})</td>
<td>-0.22</td>
</tr>
</tbody>
</table>

All samples were carried out in triplicate (n=3). \(^a\)-\(^d\) different superscripts within the same column indicate significant differences (p < 0.05)

Table 4. Texture analysis of yogurt with no bigel (Y), 10% w/w bigel (BY10), 14% w/w bigel (BY14), and 18% w/w bigel (BY18)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Firmness (g)</th>
<th>Adhesiveness (g sec)</th>
<th>Cohesiveness</th>
<th>Springiness</th>
<th>Gumminess (g sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>7.69 ± 0.88(^a)</td>
<td>-20.38 ± 7.14(^a)</td>
<td>0.72 ± 0.05(^a)</td>
<td>0.92 ± 0.02(^a)</td>
<td>5.56 ± 0.71(^a)</td>
</tr>
<tr>
<td>BY10</td>
<td>8.68 ± 0.48(^a)</td>
<td>-31.91 ± 2.66(^b)</td>
<td>0.73 ± 0.03(^ab)</td>
<td>0.93 ± 0.01(^a)</td>
<td>6.31 ± 0.28(^ab)</td>
</tr>
<tr>
<td>BY14</td>
<td>9.08 ± 0.57(^ab)</td>
<td>-36.77 ± 6.19(^bc)</td>
<td>0.71 ± 0.02(^ab)</td>
<td>0.93 ± 0.01(^a)</td>
<td>6.42 ± 0.37(^ab)</td>
</tr>
<tr>
<td>BY18</td>
<td>10.91 ± 2.18(^b)</td>
<td>-46.59 ± 7.04(^c)</td>
<td>0.64 ± 0.08(^b)</td>
<td>0.94 ± 0.01(^a)</td>
<td>7.35 ± 1.46(^b)</td>
</tr>
</tbody>
</table>

All samples were carried out in triplicate (n=3). \(^a\)-\(^c\) different superscripts within the same column indicate significant differences (p < 0.05)
Table 5. Spontaneous syneresis of yogurt with no bigel (Y), bigel with no yogurt (BG), Swiss-style yogurt (BY18) and Sundae-style yogurt (BYSU)

<table>
<thead>
<tr>
<th>Week</th>
<th>Y (%)</th>
<th>BG (%)</th>
<th>BY18 (%)</th>
<th>BYSU (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>1.21 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.66 ± 0.29&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>2.08 ± 0.03&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.12 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>2.48 ± 0.26&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.33 ± 0.23&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>4.10 ± 0.58&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.06 ± 0.02&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All samples were carried out in triplicate (n=3).
<sup>a-f</sup> different superscripts indicate significant differences (p < 0.05)

Table 6. Liquid holding capacity of yogurt with no bigel (Y), bigel with no yogurt (BG), Swiss-style yogurt (BY18) and Sundae-style yogurt (BYSU)

<table>
<thead>
<tr>
<th>Week</th>
<th>Y (%)</th>
<th>BG (%)</th>
<th>BY18 (%)</th>
<th>BYSU (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95.53 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.91 ± 0.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.24 ± 0.63&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>95.43 ± 0.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.80 ± 0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91.93 ± 0.77&lt;sup&gt;def&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>92.84 ± 0.14&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>99.44 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.34 ± 0.73&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>89.93 ± 0.44&lt;sup&gt;fgh&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>91.61 ± 0.22&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>99.03 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.94 ± 0.61&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>86.88 ± 1.22&lt;sup&gt;ij&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>90.75 ± 0.76&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>99.02 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.79 ± 0.23&lt;sup&gt;def&lt;/sup&gt;</td>
<td>85.20 ± 2.11&lt;sup&gt;ijk&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>89.43 ± 0.30&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>98.17 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.36 ± 0.87&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>85.17 ± 0.89&lt;sup&gt;ijk&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>87.94 ± 0.74&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>98.12 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.96 ± 0.30&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>83.86 ± 0.78&lt;sup&gt;k&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All samples were carried out in triplicate (n=3).
<sup>a-k</sup> different superscripts indicate significant differences (p < 0.05)
Table 7. Color parameters of yogurt with no bigel (Y), 10% w/w bigel (BY10), 14% w/w bigel (BY14), 18% w/w bigel (BY18), and bigel without yogurt (BG).

<table>
<thead>
<tr>
<th>Sample</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>C*</th>
<th>h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>69.96 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.35 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.36 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.52 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BY10</td>
<td>68.74 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.45 ± 0.03&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>8.72 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.73 ± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.52 ± 0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>BY14</td>
<td>68.94 ± 0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.53 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.25 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.27 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.51 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BY18</td>
<td>68.74 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.72 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.60 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.62 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.49 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>BG</td>
<td>63.71 ± 0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.27 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.54 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.70 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.43 ± 0.00&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Figure 4. Flow curve of yogurts with different bigel contents.
Figure 5. Apparent viscosity of yogurts as a function of shear rate.
Figure 6. Amplitude sweeps of yogurt with different bigel contents.
Figure 7. Oil migration in yogurt (Nile Red stains bigel phase) with no bigel (Y), 10% w/w bigel (BY10), 14% w/w bigel (BY14), 18% w/w bigel (BY18), Sundae-Style yogurt (BYSU) and bigel without yogurt (BG).
CHAPTER 4. OVERALL CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

Entrapment of *Lactobacillus acidophilus* and *Bifidobacterium* within soy lecithin-stearic acid and whey protein bigels significantly improved their viability in yogurt compare to free bacteria. It was very likely that the micro- and nano-structure presented in bigel entrapped the probiotics, which protected the probiotics from unfavorable conditions during yogurt storage. The incorporation of bigel in yogurt also changed the rheological properties of yogurt, such as increased viscosity, thixotropy, and yield stress. The increased firmness, reduced spontaneous syneresis, and enhanced liquid holding capacity of yogurt were observed after bigel incorporation, which might because the interactions of micro- and nano-structures of bigel with proteins in yogurt strengthened the gel structure of yogurt.

To further investigate the entrapment of probiotics in bigels before and after agitation, as well as to better understand the interaction of bigel with yogurt, microscopic analysis such as scanning electron microscope analysis should be conducted in the future. Sensory tests such as descriptive analysis by trained panelists and consumer acceptance tests will give more information about the difference between the yogurt with bigel and commercial yogurt, including the flavor profile, mouthfeel, and acceptance. *In vitro* studies of the yogurt with bigel incorporation were necessary to investigate the digestibility of this product.
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