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## Research Article

# Improving the Quality of Set Yoghurt Using Milk Fat Globule Membrane Fragments

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Lacprodan®PL20, a dairy ingredient that is rich in protein and polar lipids, was added into set yoghurts produced from nonhomogenized raw milk. The set yoghurts were prepared using concentrations of 2%, 4%, and 6% Lacprodan®PL20, while the control sample was only supplemented with skim milk powder. The effect of Lacprodan®PL20 concentrations on the physical and chemical properties, rheology, and microstructure of set yoghurt was thoroughly investigated to determine some likely improvement or changes in quality. Consequently, Lacprodan®PL20 showed a gradual improvement in the set yoghurt nutritive values, water holding capacity, and apparent viscosity. The results indicated that the firmness of set yoghurt was altered which steadily improved the gel strength, especially at 4% and 6% concentrations. The fermentation process was slightly delayed at 4% and 6% concentrations and pH values were raised as Lacprodan®PL20 concentration increased. The microstructures of the set yoghurts produced with Lacprodan®PL20, as examined by scanning electron microscopy, revealed compacted structures with fewer and smaller holes in the gel matrices. Also, a slight color change was observed in set yoghurt using a colorimeter. These results vividly showed that Lacprodan®PL20, an enriched milk fat globule membrane fragment, has the potential to improve set yoghurt quality by reducing some defects associated with set yoghurt, such as low gel strength, low dry solids, and the likes.

## 1. Introduction

The discovery and emergence of milk fat globule membrane (MFGM) in the last two decades have increasingly drawn the attention of many researchers around the world due to its wide range of applications, extraction, isolation, and purification techniques. The MFGM is extracted from dairy products, such as buttermilk, whole milk, buttermilk whey, butter serum, and acid butter through membrane filtration [1–3] or thermos-calcic aggregation [4]. The membrane surrounding the neutral triglyceride (inner core) consists mainly of polar lipids (phospholipids and sphingolipids) and specific proteins, such as glycoprotein. MFGM also consists of some

minor components, such as cholesterol, cerebrosides, water, carotenoid, iron, copper, and monoglycerides [5]. The polar lipids possess amphiphilic properties, i.e., consist of the hydrophobic tail and hydrophilic head functional groups. The lipid compositions of MFGM are predominantly triglyceride and polar lipids [6] and some of these polar lipids are identified as phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM), phosphatidylinositol (PI), phosphatidylserine (PS), glucosylceramide (GluCer), lactosylceramide (LacCer), and some few gangliosides (Gang) [4, 7]. Moreover, several proteins are found in the membrane between 25 and 70% of the membrane mass [7–9], usually characterized by sodium dodecyl sulfate

(SDS)-PAGE. The major proteins from the top of SDS gels are the mucin MUC 1, the redox enzyme xanthine oxidase/dehydrogenase, a glycoprotein PAS III, CD36, butyrophilin, glycosylated variants of periodic acid/Schiff, PAS 6/7, adipophilin, and fatty acid-binding protein [4, 8]. For instance, a recent study showed that higher amounts of peripheral MFGM proteins were found in buttermilk (BM, ~0.9 g/L) and butter serum (2.7 g/L), in comparison to integral MFGM proteins (0.3–0.5 g/L). On the other hand, a higher amount of PAS 6/7 (about 0.85 g/L) was found in buttermilk samples in compared to XO/XDH (approximately 0.50 g/L) and BTN (~0.35 g/L), i.e., BM contains approximately 5% MFGM proteins [10]. Quantitative proteome analysis of MFGM proteins using different techniques, such as Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Parallel reaction monitoring (PRM), and other techniques revealed a very high amount of MFGM proteins (approximately 950) from donkey milk and other milk, including some new abundant MFGM proteins: milk fat globule-EGF factor 8 protein, lysozyme, and polymeric immunoglobulin receptor, etc. [11, 12], with huge differences in the abundance of proteins and polar lipids identified in six commercial MFGM products [13].

Set yoghurts are generally different from other yoghurts in terms of their processing techniques, composition, and total solid and coagulum formation (fermented in the container). The microstructure and the rheological properties of set yoghurts are considered critical to product quality and shelf life. The process of serum expulsion from the gel matrix during wheying off is regarded as a technological defect in set yoghurts [14, 15]. The combination of MFGM with milk to produce yoghurt provides technological functionality in order to eliminate some common problems in yoghurt production, which include syneresis, low gel strength, poor appearances, and taste changes in set yoghurts [16–18].

Although the application of milk fat globule membrane materials to set yoghurt has been performed by a few researchers, Lacprodan®PL20 application with different concentrations to set yoghurt is yet to be studied. Therefore, this study is aimed at evaluating the functionality of different Lacprodan®PL20 concentrations, an enriched MFGM fragment, to predominantly improve the quality of set yoghurts; physicochemical properties, rheology, and microstructure.

## 2. Interpretive Summary

This research project addresses some industrial needs in providing a quality ingredient from low-cost dairy by-products. The application of enriched milk fat globule membrane (MFGM) fragment (Lacprodan®PL20) to food provides some technological functionalities to food matrix and adding nutritive values to set yoghurt by potentially supplying some health benefits to human body without causing health risks. Also, this study has important practical and economic impacts as it can reduce the production costs associated with buying conventional ingredients with limited functions and values, which are deemed objectionable. In summary, consumption of set yoghurt with an enriched

MFGM will improve consumer health benefits, confidence, and preference.

**2.1. Novelty Impact Statement.** Raising Lacprodan®PL20 concentrations to 6% considerably reinforced set yoghurt structure, chemical composition, firmness, and total solids. While presence of many specific proteins—XO and BTN—in set yoghurt supplemented with MFGM provided different changes to the quality, proteomic studies could potentially inform us of any changes to these proteins that will boost human health and improve industrial production of set yoghurt with Lacprodan®PL20.

## 3. Materials and Methods

Raw milk (pH 6.7) was collected from a farm in Mekong Delta region of Vietnam and stored in sterile clean containers at a low temperature (4°C). Skim milk powder and yoghurt starter culture were products of Asia Saigon Food Ingredients, Ho Chi Minh City, Vietnam. The culture has an equal ratio of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, (ratio 1:1). It was stored below -18°C. Lacprodan®PL20, an enriched MFGM, a spray-dried powder rich in milk phospholipids and proteins, was kindly provided by Arla Foods (Viby, Denmark). The Lacprodan®PL20 production procedures are briefly explained by Ibitoye et al. [19].

**3.1. Set Yoghurt Preparation Techniques.** Set yoghurts were prepared from nonhomogenized raw milk using starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) with three replicates. The control sample was standardized using skim milk powder only, while 2%, 4%, and 6% (w/v) Lacprodan®PL20 were added to raw milk to produce set yoghurts with 15% total solids. The supplementation of raw milk with various Lacprodan®PL20 concentrations was to replace the skim milk powder. The formulations were thoroughly mixed (for 1 min at 1000 rpm), stored overnight at 4°C in a refrigerator for complete hydration of Lacprodan®PL20, and pasteurized at 85°C for 30 min in a water vat and cooled to 42°C before inoculation with <0.05% (w/v) starter culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) from Asia Saigon Food Ingredients. The fermentation process was terminated as soon as the pH reached 4.6. The set yoghurts were immediately cooled down and stored at 4°C for one day before analyzing the components [19]. This experiment was performed three times.

**3.2. Chemical Analysis.** While the total protein content of the set yoghurts was determined by Kjeldahl's method using 6.38 as the conversion factor [20], the total lipid content was quantified using the Röse-Gottlieb method (IDF 1986). The dry matter content was determined after drying in the oven to a constant weight at 105°C. Ash content was determined by burning the samples in a furnace at 550°C. The lactose content of the set yoghurts was determined by difference [20, 21]. The measurement of total acidity was done according to the method described by Bradley et al. [22].

TABLE 1: Composition of the experiment materials expressed as % on dry-matter basis.

Materials	Total protein	Lactose	Fat content	Ash content	Dry matter	Nonfat solids
Raw milk	29.55 ± 0.07	24.96 ± 0.04	39.61 ± 0.04	5.88 ± 0.01	12.42 ± 0.27	7.50 ± 0.06
SMP	32.91 ± 0.02	54.09 ± 0.21	1.85 ± 0.14	6.83 ± 0.02	95.67 ± 0.11	93.82 ± 0.00
Lacprodan®PL20	50.34 ± 1.27	13.46 ± 0.91	29.98 ± 0.57	6.22 ± 0.70	96.4 ± 1.12	66.42 ± 0.00

The data are expressed as mean ± standard deviation of two replicates.

TABLE 2: Composition of set yoghurts expressed as % on dry-matter basis.

Lacprodan®PL20 concentration	Dry matter	Total protein	Fat	Ash	Lactose	Total acidity after fermentation (%)
Control	18.06 ± 0.13 <sup>C</sup>	31.89 ± 0.20 <sup>D</sup>	19.71 ± 0.28 <sup>C</sup>	7.81 ± 0.03 <sup>A</sup>	40.59 ± 0.24 <sup>A</sup>	1.44 ± 0.00 <sup>BC</sup>
2%	18.32 ± 0.14 <sup>BC</sup>	34.88 ± 0.08 <sup>C</sup>	19.38 ± 0.31 <sup>C</sup>	7.64 ± 0.09 <sup>AB</sup>	38.16 ± 0.06 <sup>A</sup>	1.40 ± 0.05 <sup>C</sup>
4%	18.56 ± 0.21 <sup>B</sup>	37.23 ± 0.11 <sup>B</sup>	22.41 ± 0.15 <sup>B</sup>	7.06 ± 0.04 <sup>BC</sup>	33.30 ± 0.04 <sup>B</sup>	1.49 ± 0.05 <sup>AB</sup>
6%	19.23 ± 0.29 <sup>A</sup>	38.33 ± 0.13 <sup>A</sup>	24.86 ± 0.16 <sup>A</sup>	6.71 ± 0.06 <sup>C</sup>	30.16 ± 0.06 <sup>B</sup>	1.55 ± 0.04 <sup>A</sup>

The data are expressed as mean ± standard deviation of two replicates. The means in the same column that have related superscript letters are not significantly different (Tukey's test,  $p > 0.05$ ).

The protein separation profile and all the reagents used were obtained from Invitrogen (Merelbeke, Belgium). The sample preparation was in accordance with Le et al. [23]. The wet gel was scanned at 400 dpi with a high-resolution transmission scanner (UMAX Powerlook III, Taipei, Taiwan). Protein separation was done on gradient (4-12%) polyacrylamide gels with an Xcell Surelock system. The nomenclature of the major proteins according to Mather [24] is as follows: XO, CD36, BSA, BTN, PAS 6/7, ADPH.

**3.3. pH Values Measurement.** Set yoghurt (25 mL) was taken before and after heat treatment to measure the pH value. During the incubation, the pH values were measured every hour for each sample until it reached pH 4.6 using a pH meter (Hanna, H12210, Italy). The pH meter was connected to a computer with LoggerLite program to take readings. Thereafter, the fermentation process was rapidly stopped by immersing set yoghurt in ice water for a few minutes before transferring it into the refrigerator [25].

**3.4. Firmness.** Firmness was carried out after fermentation and storage in a refrigerator at 4°C for a day. The Brookfield texture analyzer (Brookfield Engineering Laboratories, USA) was used to measure the firmness using probe TA 48. A compression test with a maximum load of 10,000 g and depth of 10 mm penetration was applied to determine the firmness. The temperature was kept constant at 5°C during the test [25].

**3.5. Determination of Water-Holding Capacity (WHC).** The WHC of set yoghurts was measured at 5,000 rpm using a centrifuge (Hermle, Z323K, Germany). A portion (25 g) of each sample was weighed into a Falcon plastic tube, covered with lids and centrifuged for 25 min at 5°C as described by Le et al. [25]. The whey was carefully removed, weighed, and calculated as follows:

$$\text{WHC (\%)} = \left[ \frac{(\text{Sample weight} - \text{Whey dispelled})}{\text{Sample weight}} \right] \times 100. \quad (1)$$

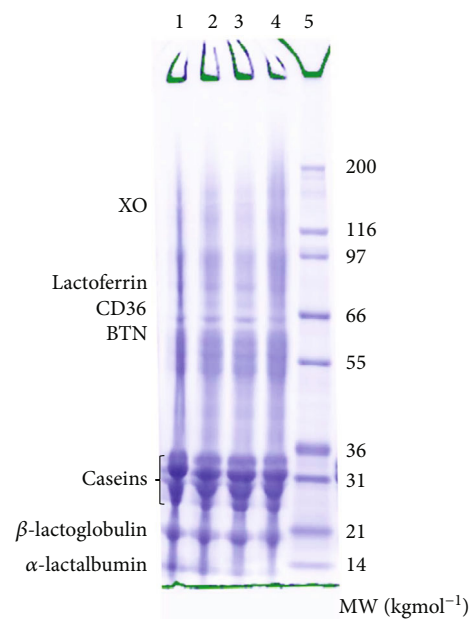


FIGURE 1: The protein profile of set yoghurts with various enriched MFGM concentrations; 6% Lacprodan®PL20 (lane 1), 4% Lacprodan®PL20 (lane 2), 2% Lacprodan®PL20 (lane 3), control sample (lane 4), and SDS-PAGE patterns of molecular weight standards (lane 5).

**3.6. Color Determination.** The color is defined by three orthogonal coordinates of  $L^*$ ,  $a^*$ , and  $b^*$ .  $L^*$  is the lightness part is from 0 (black) to 100 (white).  $a^*$  (-green to +red) and  $b^*$  (-blue to +yellow) parameters both range from -120 to 120. The set yoghurt colors were evaluated with a colorimeter (3NH, NR60CP, China) and the results were derived using CIELAB system in reference to illuminant D65 and a visual angle of 10° as reported by Pascual [26].

**3.7. Evaluation of Apparent Viscosity.** Apparent viscosity is defined as the viscosity of non-Newtonian fluid like yoghurt, which means that the apparent viscosity changes as the

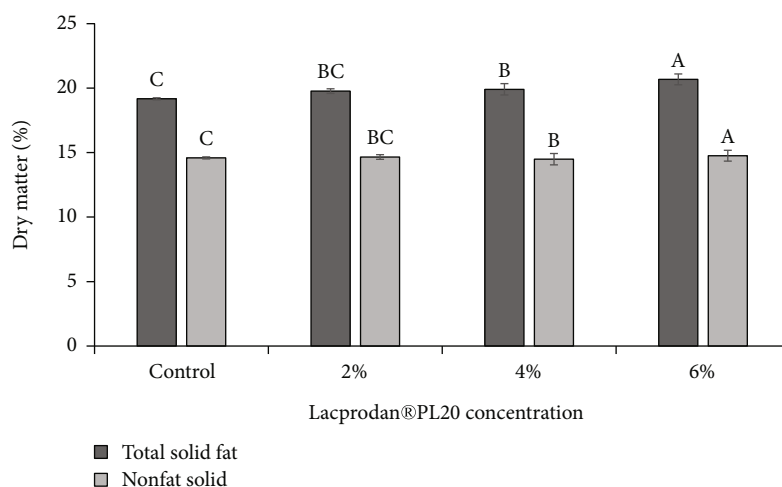


FIGURE 2: The total solid and nonfat solid of various set yoghurts produced from nonhomogenized raw milk with different levels of Lacprodan®PL20 concentration.

shear rate changes or shearing time increases [27]. The set yoghurts were gently stirred a few times in a clockwise direction with a spoon before viscosity measurements [27], then poured into a cup of height 160 mm and diameter 60 mm and subjected to a constant shear rate of  $0.5 \text{ s}^{-1}$  for 150 seconds in DV-E viscometer (Brookfield brand) equipped with the T-pieces spindle-4 to measure the viscosity at  $5^\circ\text{C}$ . A recording device was used to take the reading every 15 seconds in centipoise (cp), which was then converted to Pascal seconds (Pa.s).

**3.8. Microscopic Observation.** A scanning electron microscope (JSM 5500 made in Japan) was used to examine the various structures of the set yoghurts after incubation. Set yoghurts produced were freeze-dried at  $-96^\circ\text{C}$  for 10 hours before the examination. After that, photomicrographs were taken at  $500\times$  magnification and a  $50 \mu\text{m}$  scale bar.

**3.9. Statistical Analysis for Set Yoghurt Evaluation.** One-way Analysis of Variance (ANOVA) test was carried out to find the difference among the samples with the aid of MINITAB v16 (compatible with window 10 package) product of Minitab Inc. US (2010) and the Tukey test was used to determine the paired comparison of mean when a significant difference was observed at  $p < 0.05$ .

## 4. Result and Discussion

**4.1. Chemical Composition of Materials and Set Yoghurt.** The composition of different experimental materials is shown in Table 1. The results showed that raw milk and skim milk powder (SMP) contents conformed to the Codex standard as described [28]. On one hand, the protein and fat content of Lacprodan®PL20 were, respectively, 1.5 and 16.2 times higher than SMP protein and fat contents. On the other hand, lactose and ash content of SMP were 4.0 and 1.09 times higher than Lacprodan®PL20 contents. Also, the total solid of Lacprodan®PL20 was slightly higher than that of SMP [25, 26, 29].

The chemical composition of set yoghurts incorporated with various Lacprodan®PL20 concentrations showed a statistically significant difference in comparison with control sample ( $p$  value  $< 0.05$ ), as shown in Table 2. The fat and lactose contents of the control sample and 2% Lacprodan®PL20 concentration were not significantly different, while 4% and 6% Lacprodan®PL20 were completely different from others. The enriched set yoghurts indicated an improvement in the protein and fat contents due to the retention of MFGM fragments during membrane filtration (isolation process) resulting in total lipids and protein increase. However, a gradual decline in the lactose and ash contents as Lacprodan®PL20 concentration increased was observed [25, 26]. The membrane purification of MFGM using membrane can explain the decrease in ash and lactose contents as these can permeate through the membrane, thereby reducing the amount [1, 3].

The enriched MFGM protein profile partitioned by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is shown in Figure 1. The MFGM proteins were named according to Mather [24], starting from top-bottom, include MUCI, XO, CD36, BTN, PAS6/7, ADPH, and PP3. However, MUCI and PASIII, being glycoproteins, were not or poorly stained with Coomassie blue [24]. BTN and XO often contain about 34-43% and 13-20% of the MFGM proteins, respectively [30-32]. From the results obtained, the set yoghurts were noted to contain a high amount of  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, and caseins, which are milk proteins. Lactoferrin (glycoprotein) was present in the set yoghurt with substantial and numerous benefits such as stabilizing MFGM phospholipids vesicle, iron-binding effect, antimicrobial effects, and physiological functions [33, 34]. The set yoghurts incorporated with 6% Lacprodan®PL20 contained more MFGM proteins, especially XO and BTN, than the others. The set yoghurts with 2% and 4% Lacprodan®PL20 showed some resemblance with each other. The control sample only contained whey protein and casein and had no trace of MFGM protein. This work showed a slight improvement in the chemical

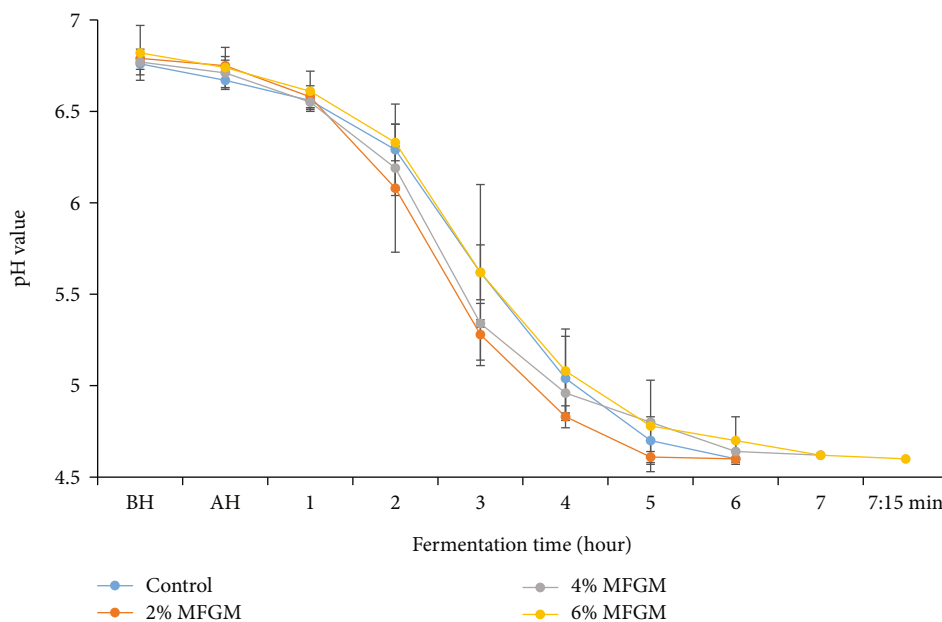


FIGURE 3: Effect of Lacprodan®PL20 on the pH values of set yoghurts produced from nonhomogenized raw milk. The control sample contains only skim milk powder, while various set yoghurts contain 2%, 4%, and 6% Lacprodan®PL20 concentrations, respectively.

composition, most especially lipid and protein contents, when compared with the previous studies [25].

Furthermore, the total solid content increased, as the SMP was replaced with 2%, 4%, and 6% Lacprodan®PL20 (Figure 2). The total solid content of 4% and 6% Lacprodan®PL20 set yoghurts was significantly higher than the control sample. On the other hand, the nonfat solids of set yoghurts prepared was statistically the same. The total acidity of set yoghurts produced after the fermentation period had some minor differences. Based on the experimental result, the total acidity of set yoghurts before and after heat treatment was the same (0.27%). A profound correlation was observed between lactose and total acidity produced (as lactose content reduced, total acidity increased portraying an inverse relationship) in Table 2. This suggested that lactose was converted by lactic acid bacteria (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) during fermentation to produce more lactic and organic acids [35]. The steady improvement in the composition points to the fact that the presence of specific proteins and polar lipids (phospholipids and sphingolipids) with other minor components of an enriched MFGM could potentially enhance the nutritive values of dairy products by effectively providing health and nutritional benefits to the consumers [4].

**4.2. pH Values Evaluation.** The influence of Lacprodan®PL20 concentration on the pH values of set yoghurt is shown in Figure 3. The results clearly showed that the pH values of set yoghurts increased as the concentration increased before heating [25] and slightly dropped after pasteurization. The slight increase among the samples before heating (BH) and after heating (AH) was a result of the difference in the ionic concentration as Lacpro-

TABLE 3: Effect of Lacprodan®PL20 concentration on the firmness of set yoghurt produce from nonhomogenized raw milk.

Lacprodan®PL20 conc.	Firmness (g)
Control	29.50 ± 0.71 <sup>AB</sup>
2%	24.50 ± 2.12 <sup>B</sup>
4%	28.00 ± 1.41 <sup>B</sup>
6%	37.00 ± 2.83 <sup>A</sup>

The data are expressed as mean ± standard deviation of two replicates of the samples. The samples in the same column that have related superscript are not significantly different (Tukey's test,  $p > 0.05$ ).

dan®PL20 concentrations increased [36]. The reduction in the pH values after heating could be due to the deposition of casein and whey protein on the surface of the MFGM fragment during pasteurization which reduced the ionic strength [2, 37, 38]. Moreover, during the fermentation period, 2% and 4% Lacprodan®PL20 concentration curves were similar from 1 to 3 hours of fermentation, whereas the control sample and 6% Lacprodan®PL20 curves were also closely related to each other within the first 4 hours of fermentation. The control sample and 2% Lacprodan®PL20 concentration curves became lower to pH 4.6 after 6 hours of fermentation. Fermentation time was prolonged for an hour with 4% Lacprodan®PL20 and 1 hour and 15 minutes with 6% Lacprodan®PL20. This might be due to an increase in the lipid level of set yoghurt as seen in Table 2.

**4.3. Firmness Result.** The experimental result of firmness vividly revealed the effect of Lacprodan®PL20 on the set yoghurts produced, as shown in Table 3. The firmness of control sample was significantly higher than the 2% and 4% Lacprodan®PL20 samples, and this is in line with the

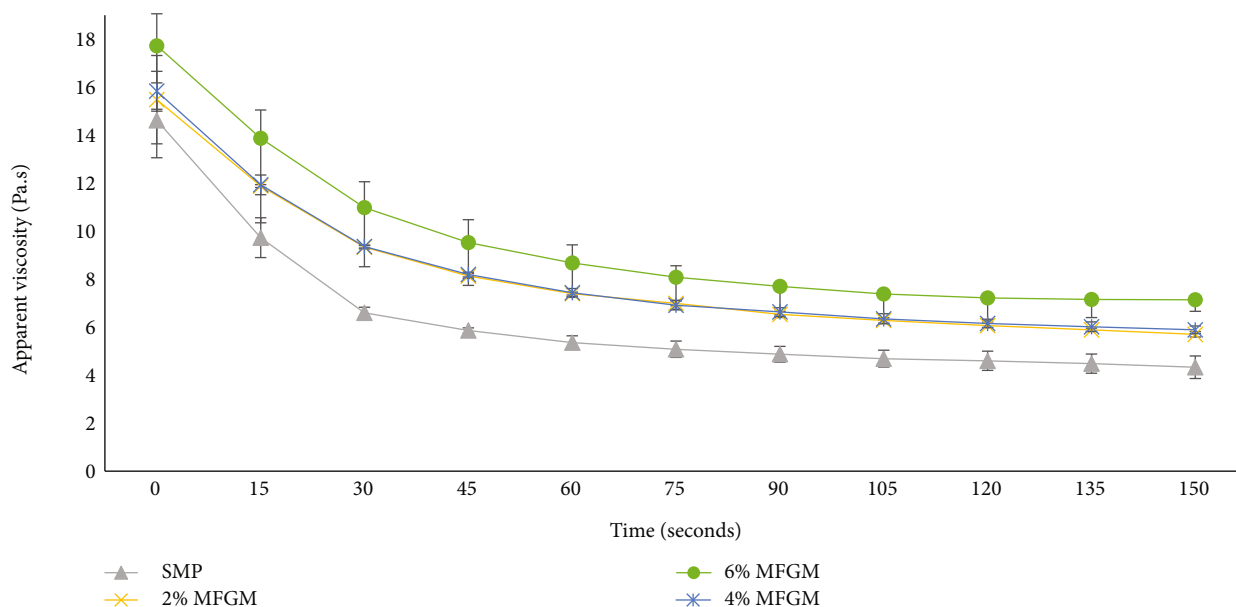


FIGURE 4: Influence of Lacprodan®PL20 on the apparent viscosity of set yoghurts produced from nonhomogenized raw milk.

experiment conducted by Le et al. [25]. However, 6% Lacprodan®PL20 resulted in the greatest firmness (37 g) due to the higher protein and fat levels (Table 2), leading to more interactions. The enriched MFGM is capable of improving the firmness of dairy products due to the specific proteins and polar lipids that were embedded in the structure [26]. Nevertheless, the weakness in the gel network of set yoghurt with 2% and 4% Lacprodan®PL20 as compared with control sample might be a result of MFGM that was unable to interact with the casein matrix. The size and volume of MFGM may also have affected the network as the large globules were likely bigger than the pores which served as a structure breaker. Another reason for this weakness is the small globules which were possibly smaller than the pores, therefore, acting as an inert filler in the gel matrix [25, 39, 40].

**4.4. Water Holding Capacity (WHC).** The water holding capacities of control sample, 2%, 4%, and 6% Lacprodan®PL20 samples were 45.85, 48.05, 51.49, and 57.46%, respectively. Replacement of skim milk powder with Lacprodan®PL20 significantly raised the water holding capacity especially with 4% and 6% Lacprodan®PL20. The gradual increase was as a result of an increase in the enriched MFGM components (protein and polar lipids) which held some water (amphiphilic properties) and consequently reduced serum expulsion (whey-off) from the set yoghurt surface [25, 26, 41, 42]. Moreover, the addition of an MFGM to yoghurt has proven to reduce whey-off drastically by stabilizing the product and maintaining the quality without any other objectionable ingredients being added, thus meeting the consumers' needs for a safe and quality food product.

**4.5. Apparent Viscosity of Set Yoghurt with Different Lacprodan®PL20 Concentrations.** The viscosity of set yoghurts produced showed evidence of thixotropic behavior (time-dependent shear thinning behavior), i.e., changes in the viscosity occurred as time function at a constant shear

TABLE 4: The initial and final apparent viscosity of set yoghurts produced from nonhomogenized raw milk with different concentrations of Lacprodan®PL20, in relation to structure lost.

Lacprodan®PL20 concentration	Apparent viscosity vs. time (Pa.s) at $0.5 \text{ s}^{-1}$		
	Initial viscosity ( $\eta_0$ )	Final viscosity ( $\eta_f$ )	Loss of structure (%)
Control	$14.62 \pm 1.56^A$	$4.33 \pm 0.47^C$	$70.39 \pm 0.04^A$
2%	$15.48 \pm 1.84^A$	$5.70 \pm 0.11^{BC}$	$62.96 \pm 3.67^A$
4%	$15.83 \pm 0.83^A$	$5.89 \pm 0.16^{AB}$	$62.71 \pm 2.95^A$
6%	$17.73 \pm 2.65^A$	$7.14 \pm 0.48^A$	$59.48 \pm 3.33^A$

The data are expressed as mean  $\pm$  standard deviation of two replicates. The set yoghurts in the same column that have related superscript letters are not significantly different, (Tukey's test,  $p > 0.05$ ).

rate (shown in Figure 4). From the results obtained, the final viscosity of set yoghurts produced was slightly different from each other with a  $p$  value  $< 0.05$ , but no statistically different in the initial viscosity [26]. The viscosity of set yoghurt increased as Lacprodan®PL20 concentrations increased. It was due to the interaction between the protein and polar lipids [18], especially the adsorption of more protein at the MFGM interface (Murray and Dickinson 1996; [29]). The initial and final viscosity of control, 2%, 4%, and 6% Lacprodan®PL20 samples, are shown in Table 4. The control sample had the lowest viscosity values, both at the initial and the final stage. The structure loss was quite fast in set yoghurts formulated with Lacprodan®PL20 compared to the control sample with no significant difference. This was also observed by Pascual [26]. Notwithstanding, more than 59-70% of set yoghurt structures were still retained after deformation caused by shearing.

**4.6. Color of Set Yoghurt Produced.** The color of the set yoghurts remained unchanged, particularly  $L^*$  and  $b^*$ ,



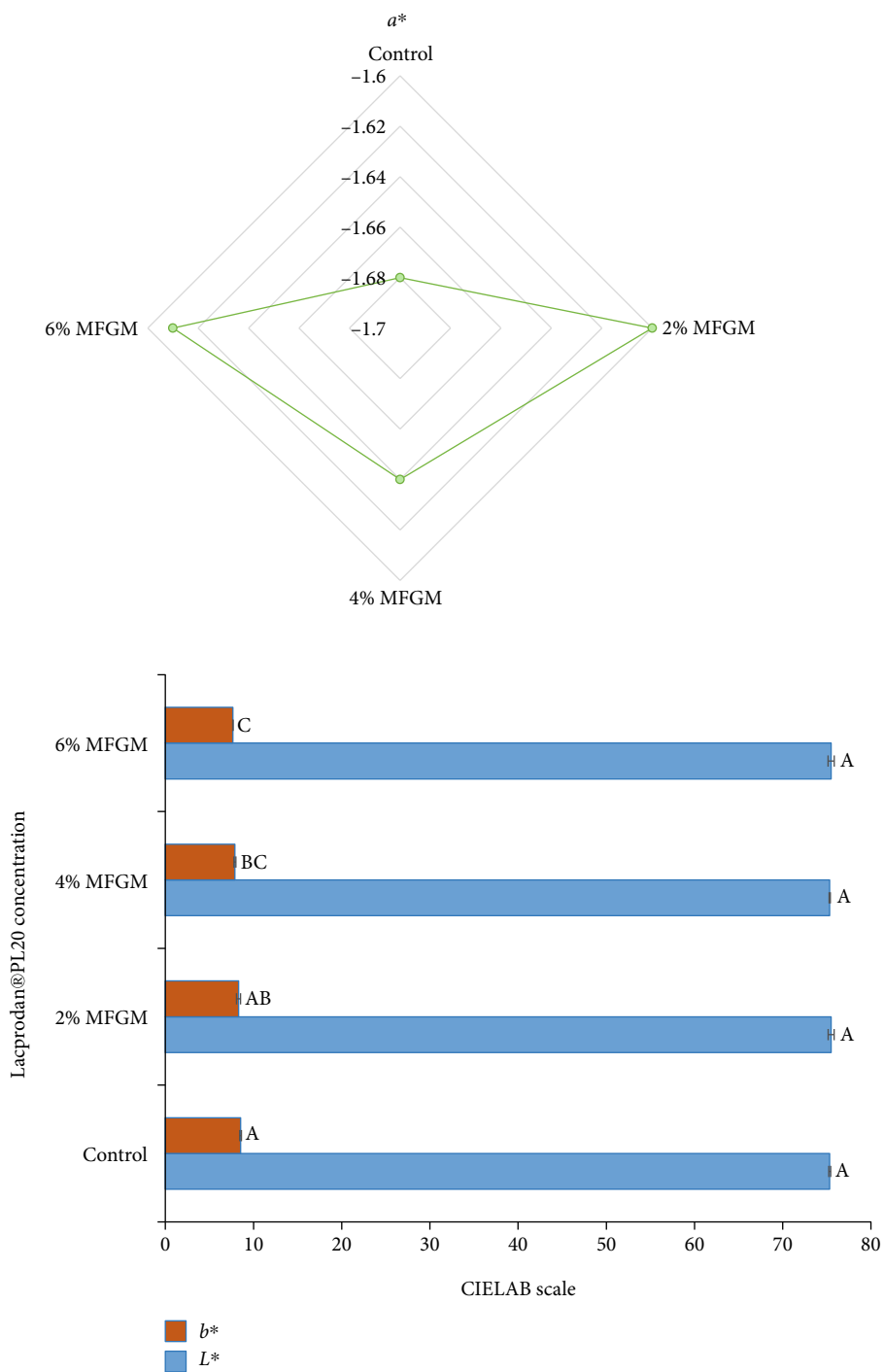


FIGURE 5: Effect of Lacprodan®PL20 concentrations on the colors of set yoghurts produced from nonhomogenized raw milk.

and only  $a^*$  had a significant difference between the set yoghurts at  $p < 0.05$ . The yellow color decreased steadily, as Lacprodan®PL20 concentration increased. This is probably due to Lacprodan®PL20 being greenish in appearance. This was more obvious in set yoghurts with 4% and 6% Lacprodan®PL20 concentration, while 2% Lacprodan®PL20 concentration was more like the control sample (Figure 5). Overall, there were not many differences in the appearance of the set yoghurts produced.

**4.7. Microstructure Examination.** The scanning electron microscopy images elucidated the interaction of enriched MFGM components with milk casein and whey protein at a microscopic level. These interactions probably include specific proteins affinity with polar lipids and other fat contents, protein to protein interaction, polar lipids, and specific proteins interaction with water. The microstructures of the set yoghurts with 4% and 6% Lacprodan®PL20 concentration displayed thicker structures with fewer and smaller openings

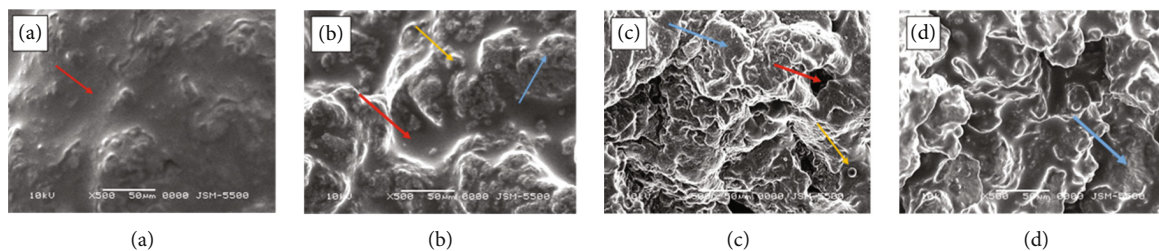


FIGURE 6: Microscopic images of set yoghurts produced with different Lacprodan®PL20 concentration. (a–d) represent 0%, 2%, 4%, and 6% Lacprodan®PL20 concentration, respectively, with 50  $\mu\text{m}$  scale bar. The yellow arrows point to the large MFGM fragments, the blue arrow shows some ruptured MFGM, and red arrows reveal the pores.

in the gel matrix (Figures 6(c) and 6(d)). The wide holes observed in Figures 6(a) and 6(b) were due to the decrease in pH during fermentation which led to the coagulation of casein proteins in set yoghurts, resulting in serum spaces filled with unprecipitated components [43–45]. In addition, some vacuums were created due to no or low amount of enriched MFGM fragments to cover up the space in Figures 6(a) and 6(b). These holes became more evident when the set yoghurts were freeze-dried, water evaporated before taking the microscopic images to keep the structure intact. Some globules in the microstructure of set yoghurt with 4% Lacprodan®PL20 were large, suggesting lack of interaction, as this could further explain a decrease in firmness (Table 3). Conversely, the microstructures of set yoghurt with 6% Lacprodan®PL20 contained a lot of ruptured fat globule membranes, showing a better interaction and the difference in the water holding capacity is clearly in support of this hypothesis. Moreover, the increase in the protein level in the 6% Lacprodan®PL20 sample (Table 2) and MFGM specific proteins (Figure 1) probably created significant interactions with each other, therefore resulting in the compacted structure that was observed in Figure 6(d). In summary, there were little interactions in both 0% and 2% Lacprodan®PL20; unlike 4% and 6%, with no or little aggregates at the surface of the gel matrix, hence creating several compatible linkages in the densely-packed structures.

## 5. Conclusion

Supplementation of Lacprodan®PL20, most especially from 4% to 6% concentrations, greatly influenced the physico-chemical properties, rheology, and microstructure of set yoghurt as studied. The authors concluded that the use of enriched MFGM material could potentially supply adequate nutritional benefits, reduced syneresis, and increase total solids and gel strengths of yoghurts. Nonetheless, there are still some enormous research opportunities yet to be investigated in the enriched MFGM, such as Lacprodan®PL20 influence on the flavor compounds of set yoghurt and sensory properties of yoghurts, especially consumer acceptability using some newly developed techniques, product shelf-life extension, and in-depth studies on MFGM and some lactic acid bacteria synergy to produce new bioactive components in yoghurt that could cure some degenerative diseases, cancers, and gastrointestinal disorder.

## Data Availability

All data are available on request from the authors.

## Conflicts of Interest

We declare that we do not have any conflict of interest.

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