International Journal of Agriculture Innovations and Research
Volume 5, Issue 6, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received: 26/04/2017 | Accepted on : 03/05/2017 | Published : 17/05/2017

Effects of Different Types of Milk Fat Globule Membrane Materials on the Physical and Rheological Characteristics of Set Yoghurts

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***Abstract* – The study on the potential applications of milk fat globule membrane (MFGM) materials as an ingredient in processed foods has become of great interest in the dairy industry today due to the functional and bioactive properties they possess. This research investigates the effects of supplementing MFGM materials from different sources, as bioactive components in yoghurt on its various physical and rheological properties such as the water holding capacity, color, flow behavior, and thixotropy. Two major experiments were conducted, the first experiment determined the chemical composition of the different MFGM materials while tests on the physical and rheological characteristics of yoghurt as influenced by the different types of MFGM materials were carried out in the second. Results revealed that different MFGM materials differ significantly in terms of their protein, fat, ash, lactose and polar lipid contents. The enrichment with different MFGM materials significantly affected both physical and rheological properties of yoghurt. Yoghurts enriched with different MFGM materials showed shear thinning and thixotropic behaviors, and increased in water holding capacity. Results of the study suggest further investigation on the effects of MFGM materials on various properties of yoghurt such as its oxidative stability and gel formation at longer storage time.**

***Keywords* – Milk Fat Globule Membrane Materials, Yoghurt, Proximate Analysis, Rheological properties**

I. Introduction

The increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for improved quality of life in their later years, has significantly changed consumer demands in the field of food production in the past few years. Today, consumers are now into functional foods, foods that are not only intended to satisfy one's hunger and provide necessary nutrients for humans but also to prevent nutrition related diseases.

Yoghurt is one of which that benefited in the rising demand for functional foods. Considered to be one of the most versatile fermented milk products, it has practically taken over the dairy aisle in many supermarkets today for its flavor and many health benefits[1].

In recent years, many researches had been geared towards the study on the composition and properties of milk fat globule membrane (MFGM). The interest in MFGM has increased considerably because of the unique polar lipids and membrane-specific proteins it contains. Sphingolipids (highly bioactive molecules, mainly present in polar lipids from animal origin) account for up to one third of the MFGM polar lipid fraction. Scientific evidence on the nutritional benefits of these sphingolipids is accumulating. It is also assumed that the MFGM proteins also possess specific nutritional properties. Moreover, MFGM material and MFGM-components have been isolated and characterized as valuable ingredients for incorporation into new food products. By means of the MFGM material, new fermented dairy products such as cheese and yoghurt with different functional and nutritional properties can be produced. This perspective could also bring economical profits by increasing the product yield or using low value by-products from the dairy industry, such as buttermilk which serves as good source of MFGM [2].

Although, various studies were already conducted on the influence of MFGM enrichment by using small fat globules on the microstructure of different cheeses [3], [4],to our knowledge, so far little work has been done to see the effects of MFGM on the sensorial and physical characteristics of yoghurt. An understanding on the textural attributes, including the desired oral viscosity of yoghurt with MFGM as bioactive components is very important for quality and consumer acceptance of the product. Controlling the gelation process is an important aspect for regulating the textural attributes of yoghurt products, hence an understanding on the structure-function relationships during fermentation and after gel is disrupted to make the yoghurt is necessary [5].

This study generally aimed to investigate the effects of supplementing MFGM materials as bioactive components in yoghurt on its various physical and rheological properties such as the flow behavior, thixotropy and gel formation.

II. Materials and Methods

Skim milk powder, buttermilk and butter serum powder (Friesland foods, Belgium); Lacprodan MFGM10-whey protein concentrate (Arla food ingredients, Denmark); Yomix 401 starter culture (Danisco, Germany); sodium citrate and citric acid (Chem-Lab, Zedelgem, Belgium) were used in the isolation of MFGM materials and manufacture of yoghurt in this study.

2.1 Preparation and Isolation of MFGM Materials

Buttermilk powder was reconstituted in deionized water with 4% buttermilk solids and 1% trisodium citrate under agitation. After the powder had completely dissolved, it was stored overnight in a refrigerator at 4°C to allow full hydration before starting the isolation. The same procedure was also followed for the reconstitution of butter serum powder. However, for the preparation of buttermilk whey samples, the reconstituted buttermilk of

8% dry solids was coagulated by the addition of citric acid (1M) to pH 4.6 at room temperature and filtered through cheese cloth. As suggested [6], before the microfiltration process pH of the reconstituted milk was adjusted to 7.5 by KOH (1M).

MFGM materials from reconstituted buttermilk, butter serum and buttermilk cheese whey were isolated using cross-flow microfiltration (MF) method in combination with three steps of diafiltration (addition of deionized water and re-concentration). The microfiltration unit consisted of a Millipore frame equipped with Pellicon 2 Cassette Filter Module, a hydrophilic PVDF multi-flat sheet membrane module with pore size of 0.22 µm and membrane surface 0.5 m^2 (Millipore, Brussels, Belgium). The microfiltration process was carried out at 45°C and pH 7.6. The final MFGM material was concentrated to about 10% solid contentand stored at < - 20°C for further analysis and yogurt making.

2.2 Chemical Analysis of the Experimental Materials

Samples of the dairy materials were examined for dry matter, fat content, protein content and ash content according to the standard procedure [7].

2.3 Yoghurt Production

Yoghurts were prepared using the Danisco yoghurt culture Yomix 401, which contains *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. Bulgaricus.* Skim milks added with the different treatments (different concentrations of MFGM materials) as specified in the succeeding experiment were heated at 85°C for 30 minutes, then later cooled to 43°C. The milks were inoculated with the working cultures and shaken well to ensure uniform distribution of the culture. Eighty (80) grams of the different mixtures were then poured to 100 grams plastic cups, capped tightly and incubated at 43°C until pH 4.6 was reached. At the end of fermentation (pH~4.6), the yoghurt gels were cooled to 5°C in ice water and stored in the refrigerator.

2.4. Yoghurts with Different MFGM Materials

In this experiment, yoghurts enriched with different MFGM materials (isolated from buttermilk, butter serum, buttermilk whey, and commercial MFGM10-lactoprodan) were prepared. Yoghurt made with 12% total dry solids from skim milk was used as control sample. A single concentration (2%) was selected for the study on the influence of different MFGM materials on the physical and rheological characteristics of yoghurt at 1st day storage. Conduct of the experiment was independently replicated three times.

2.4.1. Water holding capacity (WHC)

Centrifugation at 1250g and 5000g using Sigma 4K15 centrifuge (Buckinghamshire, England) was employed for determination of the water-holding capacity (WHC). Twenty five(25) grams (Y) of the sample was weighted into a falcon plastic tube and it was capped and centrifuged for 25min at 5°C [8]. The whey, the top layer was carefully removed and weighed (WE). The WHC expressed in percentage was calculated as using the formula:

$$WHC = \left\{\frac{Y-WE}{Y}\right\} \times 100$$

2.4.2 Color determination

The instrumental measurement of yogurt colour was carried out using a Konica Minolta CM-2500d spectrocolorimeter (Minolta Co. LTD, Tokyo, Japan) and the results were expressed in accordance with the CIELAB system with reference to illuminant D65 and a visual angle of 10°. Three determinations of each sample replicate were performed. The sample was stirred and placed in a cuvet specific for color measurement. The parameters determined were L* [L* = 0 (black) and L* = 100(white)], a* (-a* = greenness and +a* = redness), and b* (-b* = blueness and +b* = yellowness).

2.4.3. Rheological Characteristics

Test for the rheological characteristics were performed on 1st day of storage using a TA Instruments AR2000-EX controlled-stress rheometer, equipped with a concentric cylinder device consisting of a cup (30-mm diameter) and a bob (28-mm diameter) with 1.10 mm gap setting held at 5°C temperature.

For the characterization of flow curves and apparent viscosity measurements, yoghurt gels inside the cup were mixed gently by folding 8 times with cross-movements using a spatula. Samples were then passed through syringe. Yoghurt appeared to be visually homogenous after this step. Twenty (20) grams of each yoghurt samples were transferred into the rheometer cup for testing.

2.4.3.1 Characterization of Flow Curves

The shear rate was varied from 0 to 120 1/s and the shear stress and apparent viscosity were recorded at increasing shear rate (upward flow curve) followed by decreasing shear rate (downward flow curve). Both delay and constant time were set at 5s. This test was carried on duplicate samples. The temperature during the test was kept constant at 5°C. The data obtained were fitted to the power law equation.

$$\text{Shear stress} = K \text{ x } (\text{Shear rate})^n$$

Where K is the consistency index and n is the power law index. The value of n expresses the flow behavior as Newtonian (n is close to 1) or non-Newtonian (n is far from 1).

2.4.3.2 Apparent viscosity measurement

The apparent viscosity of the different yoghurt samples was measured at a constant shear rate of $60s^{-1}$ for 300s. This shear rate lies in the linear portion of the flow curve. The difference in initial apparent viscosity (η_0) and final apparent viscosity (η_e) was calculated using the formula:

$$\% \text{ Lost structure} = \frac{\eta_0 - \eta_e}{\eta_0} \times 100$$

The % lost structure is a measure of the rate of thixotropic breakdown, meanwhile the ratio of the initial to final viscosity, (η_0/η_e), can be considered as a relative measurement of the extent of thixotropy[9].

2.4.3.3. Dynamic rheological measurements

A small-strain oscillation frequency sweep was also performed on yoghurt samples at frequencies ranging from 0.1 to 10 Hz. The applied deformation was 0.1% (determined by strain sweep to be within the linear viscoelastic range). The elastic (G') and viscous (G'') moduli were recorded as a function of frequency, and the

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loss tangent, tan (δ), could then be calculated (tan δ = G''/G').

2.5. Statistical Analysis

Results from the rheological and physical characteristics determination of different yogurts were statistically processed using analysis of variance (One-way ANOVA). For significant differences, multiple comparisons of means were established with the post hoc multiple comparison-Tukey test. All statistical analyses were performed using the software Splus version 8 for Windows (Insight-ful Corp., Seatle, WA, USA) at 5% level of significance.

III. Results and Discussion

3.1 Composition of the Experimental Materials

Skim milk powder presented the lowest total fat content (1.85%) as compared to buttermilk and butter serum powder with 47.16% and 45.46% respectively (Table 1). However, it contained the highest total lactose of 55.19%. Results of the composition of buttermilk and skim milk powder were comparable with the results reported by [10]; [11].

Compositional analysis on the isolated MFGM materials on the other hand showed very highly significant difference in protein, fat, polar lipids, ash, and lactose contents. Highest protein content was recorded on the commercial MFGM10 (75.78). Whey-MFGM material revealed the lowest total protein content of (21.75%) but highest in fat (58.15%), ash (12.02%), and lactose content (14.79%). The low protein content on MFGM isolated from acid buttermilk whey was expected since large amount of protein mainly casein were already precipitated during the acid coagulation.

Table 1. Composition of the different dairy materials used in this study.

Samples*	Composition[1] On dry matter (g/100g)				
	Protein	Fat	Polar lipids	Ash	Lactose
Milk Materials:					
SMP	33.75	1.85		9.19	55.19
BMP	34.16	9.96	3.36	7.49	49.10
BSP	30.77	13.60	9.33	8.05	47.57
MFGM10-WPC	75.78	18.94	7.00	3.15	2.10
MFGM Materials:					
BM-MFGM	65.88^a± 1.47	25.50^b ± 0.50	8.43^b± 0.70	2.04^b ± 0.01	6.55^b ± 0.26
BS-MFGM	61.51^a± 1.83	28.11^b ± 1.80	23.65^a± 1.01	4.95^b± 0.19	5.41^b ± 0.39
Whey-MFGM	21.75^b± 5.75	58.15^a ± 7.06	20.97^a± 2.21	12.02^a± 9.44	14.79^a± 0.39

Values are mean of proximate composition of different samples expressed in percentage.

[a,b,c]Means for MFGM materials with the same letter (by column) are not significantly different (P>0.05)

[1]Reported data are means for duplicate batches.

*SMP=Skim milk powder, BMP=Butter milk powder, BSP=Butter serum powder

BM-MFGM=MFGM isolated from butter milk, BS-MFGM=MFGM isolated from butter serum, Whey MFGM= MFGM isolated from the acid buttermilk whey, MFGM10-WPC=commercial whey protein concentrate

Relatively high protein content was observed from MFGM materials isolated from buttermilk and butter serum. This implies that small fractions of caseins may have contaminated the final product. Casein micelles have similar size with MFGM and for such reason could give obstacle during isolation [2].

3.2 Physical and Rheological Characteristics of Yoghurts with different MFGM materials

Two percent of the isolated MFGM materials were used as the enrichment concentration in making yoghurt. From the previous test conducted, 2% MFGM was the best concentration that can be added to control formulation without severely changing the rheological properties

3.2.1. Water Holding Capacity (WHC)

WHC measurement showed a significant difference among different yoghurt treatments (Fig. 1). Highest WHC was obtained for yoghurt samples enriched with MFGM10-WPC (64.04%) while lowest was on yoghurt with whey MFGM which has 42.44%. This could be due to the difference in their protein and polar lipid contents. MFGM10-WPC was noted to contain high total protein (72%) and phospholipids (7%). The WHC of yoghurt samples enriched with BM-MFGM and BS-MFGM materials were slightly higher compared to samples with buttermilk and butter serum but the difference was not significant at p<0.05. The lower WHC or whey separation is related to an unstable gel network [12]. Insufficient amount of protein is one of the factors that favored the release of whey from the product [13]. Furthermore, an increase in the protein content increases the amount of bound water and consequently increases the firmness of the resulting gel [14].

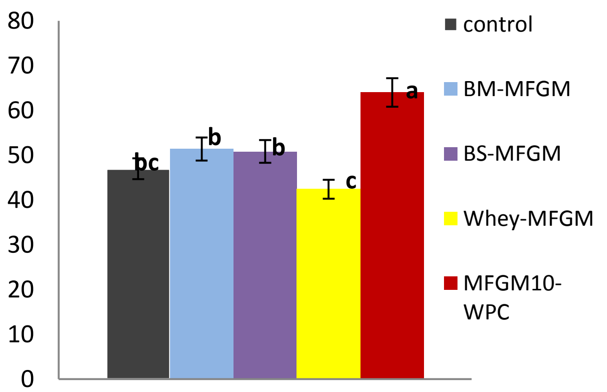

Fig. 1. Water holding capacity of yoghurt samples enriched with different MFGM materials as compared with control. Data are results of three replications. The error bar represents standard deviations. WPC with no common letters differ significantly to the Tukey's pair wise comparison (p<0.05)

3.2.2. Color Measurement

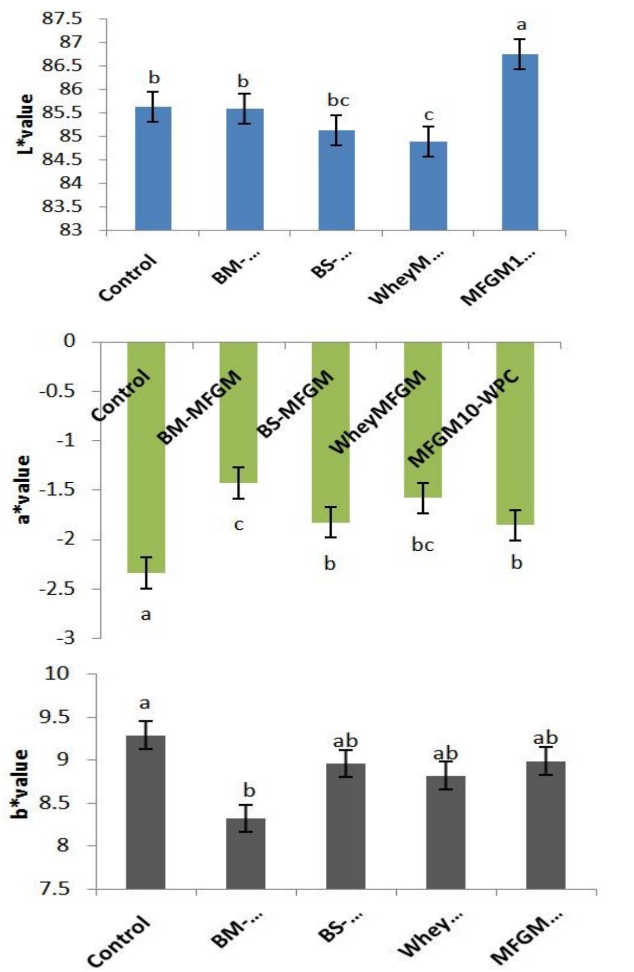

Fig. 2. Color properties of yoghurt enriched with different MFGM materials as compared with control. Data are results of three replications. The error bars represents standard deviations. WPC with no common letters differ significantly to the Tukey's pair wise comparison ($p<0.05$)

Results of the color measurement in yoghurt samples enriched with different MFGM materials as expressed according to the CIELAB system are shown in Fig. 2. Data revealed a highly significant difference in the parameter L* or brightness between yoghurt samples enriched with MFGM10 and whey MFGM. The lower L* value of yoghurt enriched with whey-MFGM could be due to the high whey expelled by the sample.

Very high significant difference was observed in a* value or green color among the different yoghurt samples with control showing the highest while yoghurt with BM-MFGM exhibited the least. In terms of parameter b*value, all the yoghurts have a yellowish component (positive b* values). Highest b* value was observed from the control, while yoghurt with added MFGM from buttermilk gave the least for yellow color.

3.2.3. Characterization of Flow Curves

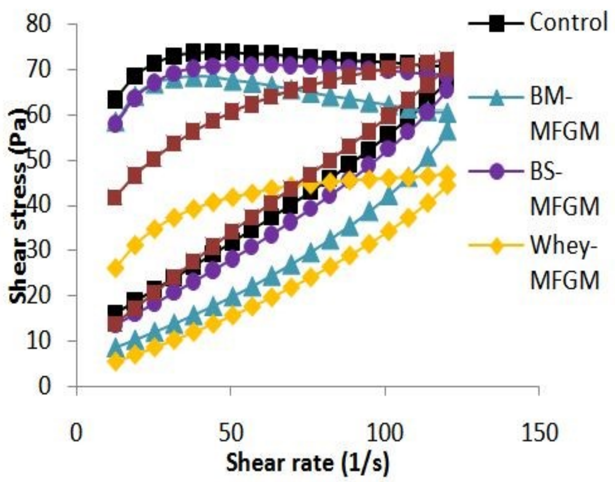

Fig. 3. Flow curves for yoghurt enriched with different MFGM Materials and control sample.

Table 2. Rheological parameters measured during flow and dynamic test for yoghurts enriched different MFGM materials and milk by-products[1]

Yoghurt	Power-law model		Parameters Apparent viscosity vs. Time (Pa.s)				Frequency Sweep	
	Consistency Index, K (Pa.s^n)	Power Law Index, n	Initial (η_0)	Final (η_e)	η_0/η_e	%Lost structure	G' (Pa)	G'' (Pa)
Control	52.91^{ab}	0.49^{a}	1.55^{ab}	0.57^{ab}	2.69^{b}	62.64^{a}	292.27^{ab}	78.02^{ab}
BM-MFGM	55.43^{ab}	0.04^{c}	1.59^{ab}	0.40^{bc}	3.92^{a}	74.32^{a}	225.03^{abc}	66.37^{abc}
BS-MFGM	52.24^{ab}	0.07^{bc}	1.52^{ab}	0.53^{ab}	2.87^{b}	65.64^{a}	245.60^{abc}	63.71^{abc}
Whey-MFGM	11.96^{c}	0.30^{ab}	0.77^{b}	0.30^{c}	2.51^{bc}	59.29^{a}	66.85^{d}	17.23^{d}
MFGM10-WPC	22.63^{b}	0.25^{bc}	1.30^{ab}	0.60^{a}	1.69^{c}	39.21^{b}	117.01^{cd}	28.84^{cd}

Control=yoghurt with 12% total solids from skim milk, BM-MFGM=yoghurt enriched with 2% buttermilk MFGM, BS-MFGM=yoghurt enriched with 2% butter serum MFGM, Whey-MFGM=yoghurt enriched with 2% whey MFGM, and MFGM10-WPC=yoghurt enriched with 2% commercial whey protein concentrate

[a,b,c]Means with the same letter (by column) are not significantly different ($P>0.05$)

[1]Reported data are means for triplicate yoghurts after 1 day storage

The enrichment of MFGM materials (BM-MFGM, BS-MFGM, and Whey-MFGM) resulted in yoghurts with lower initial shear stress as compared to the control (Fig. 3). Yoghurts enriched with BM-MFGM exhibited higher shear stress at lower shear rate but decreases constantly as shear rate increases. The complete opposite was observed in yoghurt with MFGM10-WPC, where its' shear stress increased as shear rate also increased. Among the yoghurts enriched with MFGM, materials lowest initial shear stress was observed from yoghurt samples enriched with whey-MFGM.

Except for yoghurt enriched with Whey-MFGM, significantly higher *K* values and lower *n* as compared to the control were observed from the rest of the yoghurts, indicating a thiner consistency and more deviation from Newtonian flow behavior (Table2). Based in the power-law viscosity model, consistency index *(K)* describes the reduction of viscosity as the shear rate increases which correlates to the shear thinning property. In addition, one of the major characteristics of the relationship of shear stress over shear rate was the development of a hysteresis curve; the higher the area below the curve, the higher the thixotropic effect [15] and the presence of high thixotropy is a result of more structure breakdown [16].

3.2.4. Apparent Viscosity

Results from apparent viscosity measurement of the different yoghurt samples as a function of time at constant shear rate exhibited a thixotropic behavior, as observed, viscosity decreased as time of shearing increased (Fig. 4).

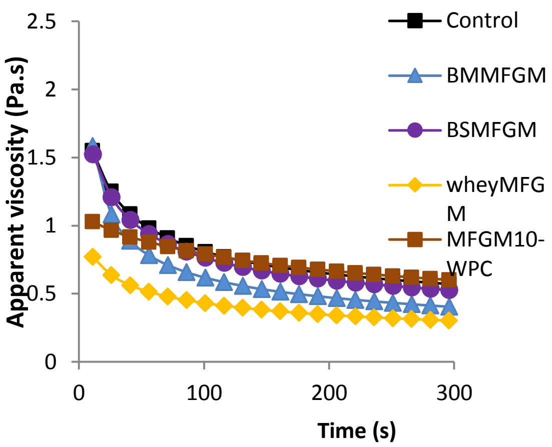

Fig. 4. Apparent viscosity vs. Time curve for yoghurt sample added with different MFGM materials and control sample after 1 day storage.

Highest initial viscosity was tallied from yoghurt with BM-MFGM (1.59) while the lowest was obtained from yoghurt sample enriched with whey-MFGM (0.77). It is also worth noting that initial viscosity of MFGM enriched yoghurts did not significantly differ from the control indicating the addition of MFGM produced yoghurt with textural properties comparable to ordinary yoghurts. After shearing, the lowest final viscosity was observed from samples enriched with MFGM10-WPC (Table2). The largest drop from initial to final viscosity on the other hand, was observed from yoghurts enriched with BM-MFGM (3.92) and BS-MFGM (2.87), while lowest was observed from yoghurts with MFGM10-WPC (1.69). This relates to higher percentage structure loss taken from yoghurts enriched with buttermilk MFGM and butter serum MFGM. As such, it can be drawn that yoghurt samples enriched with MFGM materials enhances viscosity but broken down easily when stress was applied. In a study on the exopolysaccharides in yoghurt [17], the likely reason why yoghurt samples broke down easily is that there are fewer protein-protein interaction at the critical sites (where strands are thinnest) in the network to overcome.

3.2.5. Dynamic rheological Measurements

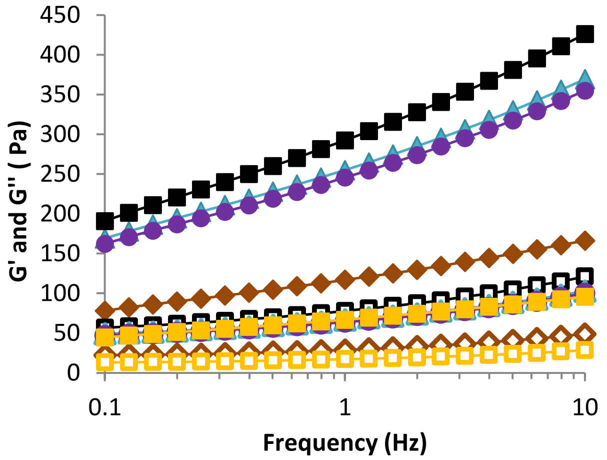

Fig. 5. Small strain oscillation frequency sweeps of yoghurts stored for 1 day at 5°C. Measurements of the elastic modulus G’ (filled shapes) and viscous modulus G’’ (empty shapes) were carried out at 5°C. The reported data are means for triplicate yoghurts and analysis. Where: Control (,), BM-MFGM (,)BS-MFGM (,),Whey-MFGM(,) and MFGM10-WPC(,)

Small-amplitude shear stress oscillatory test was conducted to study the gel texture after 1 day storage. Figure4 shows the results of the frequency sweep reported as G’ or G’’ vs. log of frequency. Straight lines for all yoghurts were observed in the range of frequencies tested. All yoghurt samples exhibited characteristics of a weak viscoelastic gel, showing greater G’ than G’’ at the frequencies investigated. Table2 shows the mean elastic storage and loss modulus determined at 1 Hz for the different yoghurts after 1 day storage. Highest G’ and G’’ were recorded from the control while the lowest was observed from samples enriched with whey MFGM. However, values for G’ and G’’ of yoghurts enriched with different MFGM (BM-MFGM and BS-MFGM) materials did not significantly differ from the control.

IV. Conclusion

The different isolated MFGM materials differ significantly in terms of their protein, fat, ash, lactose and polar lipid contents. The enrichment with different MFGM materials significantly affected both physical and rheological properties of yoghurt. Yoghurts enriched with different MFGM materials showed shear thinning and thixotropic behaviors. Moreover, samples with MFGM materials isolated from buttermilk (BM-MFGM) and butter serum powder (BS-MFGM) exhibited higher structure loss. The addition of MFGM materials however, basically increased the water holding capacity of yoghurt. The brightness of yoghurts enriched with MFGM isolated from buttermilk and butter serum did not significantly differ from the control. In terms of their viscoelastic properties, samples enriched with Whey-MFGM produced the weakest gel.

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