International Food Research Journal 30(2): 334 - 342 (April 2023)
Journal homepage: *http://www.ifrj.upm.edu.my*

Emulsification potential of milk fat globule membrane material microfiltrated from buttermilk whey

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Article history

Received:
25 June 2021
Received in revised form:
22 March 2022
Accepted:
15 July 2022

Keywords

dairy side stream,
microfiltration,
MFGM,
emulsion

Abstract

The emulsification potential of milk fat globule membrane (MFGM) material obtained from buttermilk whey was investigated. A microfiltration technique was applied to recover MFGM material from the whey, a side-stream of the cheese-making process from the buttermilk. During the preparation of O/W emulsions, a constant ratio of protein and oil was maintained, and homogenised at 0/2, 3/2, 9/2, and 15/2 MPa pressures using a two-step homogeniser. Emulsions prepared with buttermilk powder (BMP) and microfiltrated buttermilk whey (MFBMW) showed similar microstructure and rheological properties. The particle distribution and mean diameter ($D_{3,2}$) were similar for both materials. However, no cream separation was observed for emulsion prepared with MFBMW during 8-day storage. Despite the differences in the composition, MFBMW showed good creaming stability, and similar emulsifying properties to BMP. These results demonstrated the future perspective of whey valorisation in a high food value dairy application.

DOI
https://doi.org/10.47836/ifrj.30.2.05

Introduction

The complex biological membrane covering the fat globules in milk, known as the milk fat globule membrane (MFGM), has an interesting technological functionality due to its composition of unique membrane proteins and polar lipids. The amphiphilic nature of such membrane materials defines the emulsifying properties of the MFGM. The membrane materials collected from milk fat globules have a great potential for use as emulsifiers or stabilisers in food systems (Dewettinck *et al*., 2008; He *et al*., 2017). The physicochemical characteristics of dairy emulsions are greatly influenced by their milk-derived components. Several dairy ingredients such as powder from skim milk or buttermilk, whey protein, casein, and isolated phospholipids have been well studied in the production of emulsions (Lazzaro *et al*., 2017; Shen *et al*., 2017; Ali, 2019).

In recent years, growing attention has been paid to the potential of MFGM material due to its nutritional and unique functional properties (He *et al*., 2017; Lopez *et al*., 2017). In raw milk, such a naturally occurring membrane prevents the flocculation, aggregation, and coalescence of the dispersed fat globules, and protects against enzymatic actions. However, the functional properties and protective measures of MFGM are influenced mainly by the kinetic conditions during processing such as pumping, heating, and homogenisation (Evers, 2004; Lopez *et al*., 2019). The technological functionalities of the recovered MFGM material also depend on the isolation method (microfiltration, ultracentrifugation, *etc*.), process condition (temperature, pH, Ca^{++}, *etc*.),

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and raw material (raw milk, buttermilk, whey, *etc*.) quality (Singh, 2006; Ward *et al*., 2006; Le *et al*., 2009; Abd El-Salam and El-Shibiny, 2020). For instance, the MFGM isolated from fresh cream had superior emulsifying properties than that separated from dairy by-products (Corredig and Dalgleish, 1997; Wong and Kitts, 2003). Other researchers have also reported that the MFGM from buttermilk had effective emulsifying properties (Roesch *et al*., 2004; Ali, 2019; Jukkola *et al*., 2019). Phan *et al*. (2013) reported that the MFMG materials microfiltrated from reconstituted buttermilk had better emulsifying capacity than those recovered from skim milk powder (SMP), BMP, and sodium caseinate.

Dairy side-stream products such as whey from the cheese-making process using buttermilk are generally considered inferior by-products. Proper utilisation of this side-stream through isolation of the functional MFGM materials, and application in the food system will add value to the food industry (Panghal *et al.,* 2018). Buttermilk whey (BMW) contains whey proteins, bioactive peptides, and fat residue rich in lipoprotein particles and MFGM materials (Rombaut *et al*., 2007; Barukčić *et al*., 2019). Hence, the buttermilk whey could be a source of MFGM material for application in added-value food processing. To the best of our knowledge, a number of studies have been reported describing the emulsification potential of MFGM materials microfiltrated from buttermilk (Corredig and Dalgleish, 1997; Roesch *et al.*, 2004; Phan *et al.*, 2014; 2020; Jukkola *et al*., 2019; Chen *et al*., 2020). Phan *et al*. (2014) have compared the emulsification properties of commercial and extracted MFGM and their blend. The authors applied a homogenisation pressure of 9/2 MPa to prepare the emulsions. However, a pressure-dependent emulsification potential MFGM material isolated from buttermilk whey needs to be further elucidated.

Therefore, the present work investigated the emulsification potential of the MFGM materials isolated from the buttermilk whey. The microfiltration technique was applied to isolate the MFGM materials, and four different combinations of homogenisation pressure were applied to prepare the emulsion. The capacity of MFBMW to stabilise the oil-in-water (O/W) emulsion in terms of emulsion structure, stability, particle size distribution, and rheological behaviour was compared with buttermilk powder.

Materials and methods

Materials

Chemicals for analyses were purchased from Sigma Aldrich (Germany) or Chem-Lab (Belgium). HPLC-grade chemicals were purchased from Biosolve (Netherland). Deionised water was obtained from the laboratory water purification system (Millipore SA, France). Buttermilk whey was provided by the Böllinger Butterei (Belgium), and buttermilk powder from Friesland-Campina (Belgium)

Isolation of the MFGM materials

The pH of the buttermilk whey was adjusted to 7.5 by adding the required amount of KOH (1.0N) (Rombaut *et al.,* 2007). The isolation of MFGM material was performed using a cross-flow microfiltration technique containing a Millipore frame with two cassette filters (PVGVPPC05), and 0.22 µm pore size PVDF hydrophilic membrane (Durapore®) with a surface of 0.5 m^2 (Screen type C). A feed pump (Chemicor Series of Almatec, Kamp-Lintfort, Germany) with a flow rate of 200 L/h, a peristaltic pump (Millipore, SA, France) with a permeate flow rate of 15 L/h, and the trans-membrane pressure of 0.35 - 0.55 bar were adjusted. A four-step continuous diafiltration was performed at 40 - 45°C. The final MFGM material was stored below -20°C for the preparation and characterisation of the emulsion.

Compositional analysis of the experimental materials

Dry matter, total protein, total fat, ash, and lactose contents were determined according to Phan *et al.* (2013). Polar lipids were obtained by solvent extraction method and analysed with an HPLC system (Shimadzu, Japan) connected with an ELSD detector (Alltech-3300, Alltech Associates Inc., Belgium) according to Le *et al.* (2011). A pre-column (7.5 × 3.0 mm; Prevail silica, 5 µm) and separation column (150 × 3.0 mm; Prevail silica, 3 µm) were used with a column oven temperature of 40°C. The sample injection volume was 10 µL, and all samples were analysed in triplicate.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to determine the protein profile of the sample materials. The separation of different proteins was based on the molecular mobility in the acrylamide gel matrix depending on their molecular weight. The SDS-

PAGE method of protein determination and calculation was according to Phan *et al.* (2013).

Preparation of emulsion

Isolates from microfiltrated buttermilk whey and BMP were used for emulsion preparation. Before emulsion preparation, BMP was hydrated overnight at 4°C. Oil-in-water emulsions were prepared with 35% soy oil, and the pH was adjusted to 7.0. For all emulsions, protein content was maintained at 2.3 g per 100 g. The mix was initially homogenised at 50°C with the Ultra-Taurrax mixer (IKA, Germany) for 2 min at 13,000 rpm. Finally, the mixtures were homogenised at four different homogenisation pressures (0/2, 3/2, 9/2, and 15/2 MPa) using a two-step homogeniser (APV, Denmark). All emulsions were then stored at 4°C for 24 h.

Particle size measurement

The particle size distribution of the emulsion samples was measured using a Mastersizer (Malvern instrument, UK) equipped with a 300RF lens. All samples were measured either by dispersing in water or 1% sodium dodecyl sulphate solution according to Phan *et al.* 2013. For sample analysis, the particle refractive, absorption, and dispersant refractive index were set at 1.5295, 0.01, and 1.3300, respectively (Roesch *et al.*, 2004). The surface-volume mean diameter ($D_{3,2}$) was used to compare the droplet size of the samples. All samples were measured in triplicate.

Microscopic observation

For microscopic evaluation, all samples were diluted ten times in deionised water. A small drop of the sample was placed on a glass side, and covered with a coverslip to prevent dehydration. The emulsion morphology was observed at 50× magnification under the microscope (Leitz Diaplan Leica, Germany). Sample images were taken using a built-in camera (Olympus, Aartselaar, Belgium), and further processed using cell D software (Phan *et al.*, 2013).

Emulsion stability

Immediately after homogenisation, 10 mL of each emulsion sample was transferred into graduated glass tubes of 10 mm diameter, and stored at 4°C for 8 d. On alternate days, the volume of the serum layer accumulated at the bottom of the graduated tube was recorded. All samples were measured in triplicate. The percentage of separation was calculated using Eq. 1:

$$\%\ Serum\ seperation = \frac{Volume\ of\ serum}{Volume\ of\ emulsion} \times 100 \quad \text{(Eq. 1)}$$

Rheological characteristic

Rheological measurement was performed using a controlled-stress rheometer (AR2000, TA instrument, USA). The measuring geometry consisted of a conical cylinder (28 mm, concentric) and a sample holding cup (30 mm diameter). Briefly, gently mixed 20 g emulsion samples were poured into the sample cup, and the flow curves were obtained by applying increasing shear rates (0.1-100 s^{-1}) which provided 31 measuring points. The measurement temperature was maintained at 20°C, and all samples were measured in triplicates. The data were fitted to the Power-Law model as shown in Eq. 2:

$$Shear\ stress = K \times Shear\ rate^{n} \quad \text{(Eq. 2)}$$

where, K = consistency index, and n = flow behaviour index.

Statistical analysis

Statistical analysis was performed using XLSTAT version 2020.1.3 for Windows (Addinsoft, Milan, Italy). Tukey's Honest Significance Test was used to compare mean values at $p \leq 0.05$ significance level.

Results and discussion

Chemical composition of the experimental materials

The moisture content of MFBMW and BMP was 7.34 and 95.77%, respectively. The total protein, total lipid, lactose, ash, and polar lipid contents of MFBMW were 22.78, 28.39, 39.27, 9.56, and 13.15%, respectively (dry matter basis). In BMP, they were 34.28, 8.27, 50.27, 7.23, and 3.26%, respectively. A significant ($p \leq 0.05$) difference was observed between the experimental materials. The MFBMW contained 1.5- and 1.3-times lower protein and lactose contents, respectively, but 3.4-, 1.3-, and 4.0-times higher lipid, ash, and polar lipid contents, respectively, as compared to BMP. These results are in agreement with the findings of other authors (Le *et al.*, 2011; Phan *et al.*, 2013). However, the lipid content of our MFBMW sample was lower than reported in the previous studies. This might have been

due to the batch-to-batch variation of the microfiltration process. Unlike BMP, the higher polar lipid content of MFBMW indicated the enrichment of MFGM material. Due to the damaged fat globule membrane, high polar lipid content was recovered as retentate of the microfiltration process. The addition of $CaCl_2$ during the cheese-making process might be the source of higher ash content in MFBMW. The lower lactose in MFBMW was expected because a portion of lactose was drained out during the microfiltration process (Rombaut *et al.*, 2007).

The SDS-PAGE of dairy materials was performed to understand the protein composition qualitatively. Figure 1 shows the representative SDS-PAGE profile of the experimental samples.

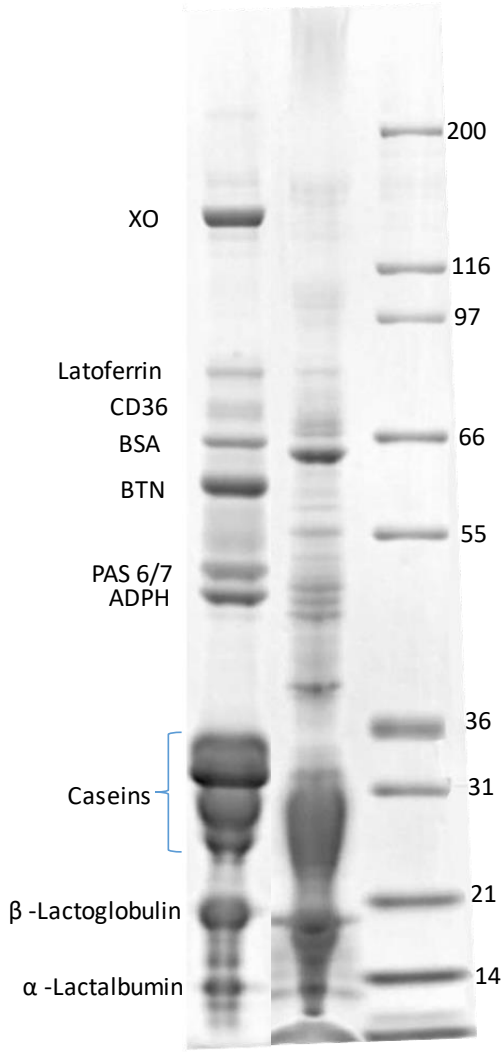

Figure 1. SDS-PAGE electrophoretograms of dairy materials: line 1 = buttermilk powder (MBP); line 2 = microfiltrated buttermilk whey (MFBMW); and line 3 = molecular mass (kDa) standards. The load of each line contained 12 µg total protein. XO = xanthine oxidase; BSA = bovine serum albumin; BTN = butyrophilin; and ADPH = adipophilin.

The band of well-reported MFGM proteins (Singh, 2006) was present in a considerable amount in buttermilk powder (line 1) but weakly present (*e.g.* BTN, PAS6/7, ADPH) or even absent (*e.g.* lactoferrin, XO, CD36) in MFBMW (lane 2). However, a considerable amount of casein, β-lactoglobulin, and a small portion of α-lactalbumin was found in MFBMW. It is possible that casein micelles, having a similar size to MFBMW, might be isolated as retentate (Rombaut *et al.*, 2007).

Distribution of the particle size in the emulsion

Figure 2 shows the average particle size distribution in emulsions prepared from MFBMW and BMP at different homogenisation pressures of 0/20, 30/20, 90/20, and 150/20 MPa. A different particle size distribution was observed when the measurement was performed after diluting in 1% SDS solution as compared to water (Figure 2 and Table 1). Both materials showed bimodal particle size distribution when measured after diluting in water. There was a small second peak around 0.1 to 1 µm range, but the major volume fraction was about 1 - 100 µM range (Figure 2, bottom). This might be the consequence of bridging, flocculation, or clustering of the fat globules, meaning that newly formed particle surfaces might be partially covered, and shared a common protein at the interface with neighbouring particles (Dickinson, 2017). Whereas in 1% SDS solution, the distribution pattern was monomodal; narrower size range and slight skewness were evident on the left side (Figure 2, bottom) for emulsion prepared at homogenisation pressure < 15/2 MPa. Skewness on the left side indicated the contribution of smaller particles in the emulsion, and the estimation of mean particle size might be influenced due to the nature of the underlying data. However, at 15/2 MPa, emulsion showed normal distribution without skewness, which indicated that the mean particle size was not under/overestimated due to the presence of outliers.

BMP contained higher total protein and casein fractions than MFBMW (Figure 1 and chemical composition section). It is well known that the emulsifying potential of a dairy ingredient depends on the aggregation behaviour of the component proteins. For instance, self-aggregation of casein strongly influences emulsifying behaviour, and is considered as a less efficient emulsifier (Lazzaro *et al.*, 2017). Instead, MFBMW was rich in polar lipids, which could increase its emulsifying properties. The

MFBMW had high ash content (*e.g.* Ca^{2+} due to adding $CaCl_2$ during cheese making). Calcium being a divalent cation, can easily bridge casein or two negatively-charged groups of protein (Rombaut *et al.*, 2007). This can give rise to protein aggregation, and subsequently a bimodal particle distribution in emulsions when the measurement was done after dilution in water.

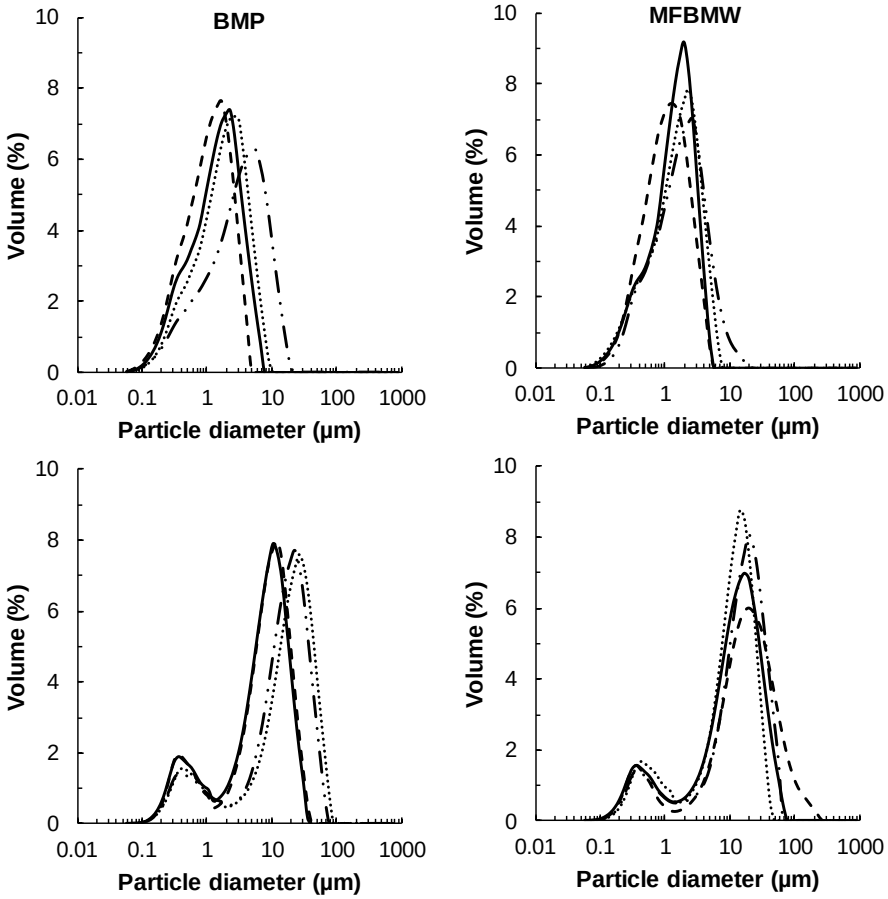

Figure 2. Particle size distribution of emulsions prepared with BMP and MFBUMW at different homogenisation pressures (— ·· — = 0/2; ····· = 3/2; —— = 9/2; and — — = 15/2 MPa). Measurements were carried out after dilution in 1% SDS (top) or water (bottom). Data are means of three independent replicates (n = 3).

For both materials, the $D_{3,2}$ values of emulsions decreased significantly ($p < 0.05$) with increasing homogenisation pressure after dilution in 1% SDS, but no significant reduction of $D_{3,2}$ values was observed when the emulsions were diluted in the water. The average particle diameter in both materials was similar (Table 1). Adding SDS further improved the stability because SDS disrupted particle aggregates and displacement of protein, and were easily absorbed in the oil-water interface (Goibier *et al.*, 2017). In general, MFBMW obtained from the dairy side-stream showed similar emulsifying properties as compared to BMP, the mainstream product of buttermilk. The droplet size distribution was not only influenced by the participation of casein, whey proteins, and MFGM specific proteins, but also to a greater extent by polar lipids.

Microscopic observation

Figure 3 shows the microstructure of emulsion at different homogenisation pressures. In general, emulsions prepared with MFBMW and BMP showed a similar structure at different homogenisation pressure. This result is in agreement with particle size distribution after diluting in water (Table 1). Although MFBMW contained less protein (casein and MFGM specific protein) and more minerals (*e.g.* $CaCl_2$) as compared to BMP, it showed a similar capacity of stabilising the emulsion. This might have been due to the influence of proteins and the higher proportion of polar lipids present in MFBMW (Figure 1 and composition).

Table 1. Sauter mean diameter ($D_{3,2}$) and flow curve parameters of emulsions prepared with BMP and MFBMW at different homogenisation pressures.

Pressure (MPa)	$D_{3,2}$ (µm) after dilution in water		$D_{3,2}$ (µm) after dilution in SDS		Flow curve parameter			
					BMP		MFBMW	
	BMP	MFBMW	BMP	MFBMW	*K* (Pa.s^n)	*n*	*K* (Pa.s^n)	*n*
0/2	8.33 ± 0.67^{b}	5.16 ± 0.26^{a}	3.79 ± 0.25^{d}	3.47 ± 0.42^{d}	0.13 ± 0.01^{a}	0.85 ± 0.01^{d}	0.32 ± 0.03^{a}	0.67 ± 0.03^{d}
3/2	7.67 ± 1.97^{ab}	5.59 ± 0.54^{ab}	2.68 ± 0.12^{c}	2.61 ± 0.08^{c}	0.75 ± 0.32^{b}	0.7 ± 0.09^{c}	1.33 ± 0.11^{b}	0.54 ± 0.02^{c}
9/2	6.36 ± 0.8^{a}	6.28 ± 0.23^{bc}	2.14 ± 0.09^{b}	2.11 ± 0.18^{b}	3.78 ± 1.62^{c}	0.54 ± 0.06^{b}	3.80 ± 0.76^{c}	0.44 ± 0.01^{b}
15/2	6.96 ± 1.27^{ab}	6.27 ± 0.44^{c}	1.58 ± 0.03^{a}	0.93 ± 0.20^{a}	13.90 ± 2.74^{d}	0.40 ± 0.03^{a}	9.44 ± 1.20^{d}	0.35 ± 0.02^{a}

Values are mean ± standard deviation of three replicates (n = 3). Means followed by different lowercase superscripts in a column are significantly different ($p \leq 0.05$). *K* = Consistency index; *n* = Power-Law index; MFBMW = microfiltrated buttermilk whey; BMP = buttermilk powder; and SDS = sodium dodecyl sulphate.

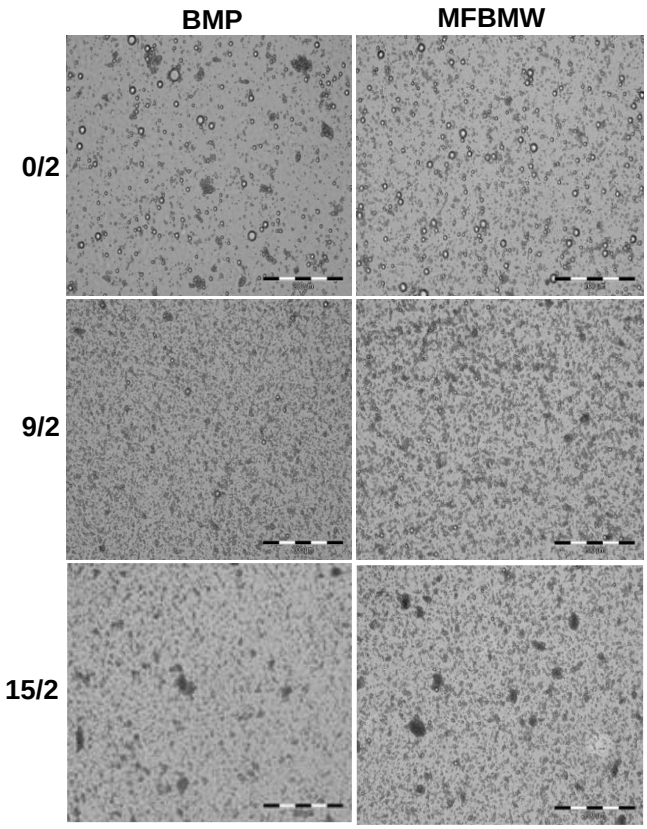

Figure 3. Microscopic images of emulsions prepared with BMP and MFBMW. Bar scale = 200 µm.

Stability of emulsion

Emulsions prepared with MFBMW showed no serum separation irrespective of homogenisation pressure. In BMP samples, no serum separation was observed for emulsions prepared at higher homogenisation pressure (≥ 9/2 MPa), but noticeable at lower pressure. The better creaming stability of emulsions prepared with MFGM material could be due to the positive effect of polar lipids (*e.g.* phospholipids) recovered during microfiltration of buttermilk whey (Phan *et al.*, 2013; He *et al.*, 2017). The hydrophobic proteins and higher content of polar lipids in MFBMW played an important role in stabilising the emulsion by covering the surface of the newly formed particles (Chen *et al.*, 2020). At higher pressures, exposure of the hydrophobic sites of the globular protein facilitates protein interaction at the surface, which results in the reduction of surface tension, and ultimately the stability of the emulsion (Tang, 2020). In general, MFBMW obtained from the dairy side-stream showed better emulsion stability as compared to buttermilk powder.

Rheological behaviour

The shear stress versus shear rate curve for BMP and MFBMW are presented in Figure 4. The data were well fitted in the Power-Law model ($p < 0.05$) with $R^2 \geq 0.998$. The model parameters are reported in Table 1. All emulsions showed a similar trend of shear-thinning behaviour. The shear stress increased gradually against shear rates. This might have been due to the fact that particle aggregates started to disintegrate under higher shear rates (Ariffin *et al.*, 2016). The results were consistent with the changes in the average particle diameter of the emulsions diluted in different materials (Table 1). For both materials, shear stress increased with increased homogenisation pressure. Both emulsions showed a shear-thinning and thixotropic property at higher homogenisation pressures (≥ 3/2 MPa). The consistency index (*K*) increased, and the flow index (*n*) decreased as homogenisation pressure increased (Table 1).

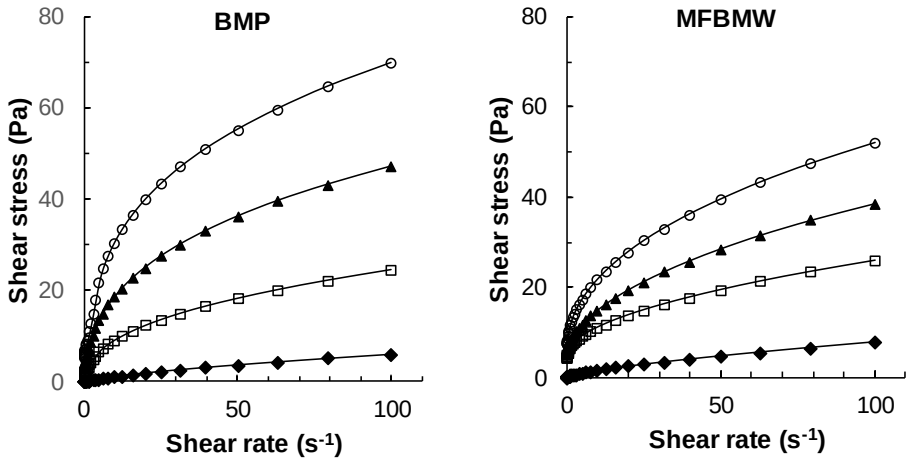

Figure 4. Flow curves of the emulsions prepared with BMP and MFBMW at different homogenisation pressures (◆ = 0/2; □ = 3/2; ▲= 9/2; and ○ = 15/2 MPa). Data are means of three independent replicates (n = 3).

Conclusion

Microfiltration facilitated the recovery of the MFGM materials from the poorly known dairy side-stream of buttermilk whey. The emulsion prepared with MFBMW and BMP showed similar microstructure and rheological properties. The particle distribution and mean diameter were also similar for both materials after diluting in either water or 1% SDS. It was expected that MFBMW would have superior emulsifying properties as it contained higher polar lipids; but, the higher mineral content minimised the effect. However, narrow particle distribution and smaller particle mean diameters were obtained in 1% SDS. In addition, no serum separation was observed during 8-day storage for emulsions prepared with MFBMW. MFBMW showed better emulsifying properties at homogenisation pressure 15/2 MPa in terms of mean particle diameter, serum separation, consistency, and flow index. In conclusion, MFBMW microfiltrated from the dairy side-stream had interesting emulsifying properties as compared to BMP. Further investigations should be made into process optimisation to reduce mineral content and exploit other technological functionalities.

Acknowledgement

The authors gratefully acknowledge Dr. Koen Dewettinck, Laboratory of Food Technology and Engineering, Ghent University, Belgium for providing laboratory facilities for the completion of the present work.

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