The effect of addition of high-melting monoacylglycerol and candelilla wax on pea and faba bean protein foam-templated oleogelation

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Abstract

Oleogels prepared from hydrocolloids have recently gained a lot of attention as an alternative for trans and saturated fats. Previously we have demonstrated that the freeze-dried foams prepared using a mixture of 5% faba bean or pea protein concentrates with 0.25% xanthan gum at pH 7 and 9 can hold canola oil 30-40 times their weights (Mohanan, Tang, Nickerson and Ghosh, 2020). However, the oleogels suffered from high oil loss, about 30% oil leaked, which negatively affected the rheological properties of the oleogels. The functionality of the cake baked using the oleogels was poorer compared to a shortening baked cake. The present study explored the addition of a small amount of high-melting monoacylglycerol (MAG) and candelilla wax (CW) on reducing oil loss, improving rheological properties and baking qualities of pulse protein-stabilized oleogels. Different concentrations (0.5-3%) of MAG or CW were dissolved in canola oil at 80 ºC. The hot oil was then added into the freeze-dried protein-polysaccharide foams (pH 7) and quickly transferred to a refrigerator. The crystallized additives reinforced the oleogel network, thereby reducing oil loss while increasing the firmness, cohesiveness, and storage modulus. When model cakes were baked with the oleogels, significant improvement in textural properties was observed with the addition of MAG in the foam-templated oleogels. However, in comparison with shortening-based cakes, oleogel-based cakes still showed a negative effect on hardness, chewiness and cohesiveness.

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Pulse protein foam-templated oleogels

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Abstract

Oleogels prepared from hydrocolloids have recently gained a lot of attention as an alternative for trans and saturated fats. Previously we have demonstrated that the freeze-dried foams prepared using a mixture of 5% faba bean or pea protein concentrates with 0.25% xanthan gum at pH 7 and 9 can hold canola oil 30-40 times their weights (Mohanan, Tang, Nickerson and Ghosh, 2020). However, the oleogels suffered from high oil loss, about 30% oil leaked, which negatively affected the rheological properties of the oleogels. The functionality of the cake baked using the oleogels was poorer compared to a shortening baked cake. The present study explored the addition of a small amount of high-melting monoacylglycerol (MAG) and candelilla wax (CW) on reducing oil loss, improving rheological properties and baking qualities of pulse protein-stabilized oleogels. Different concentrations (0.5-3%) of MAG or CW were dissolved in canola oil at 80 °C. The hot oil was then added into the freeze-dried protein-polysaccharide foams (pH 7) and quickly transferred to a refrigerator. The crystallized additives reinforced the oleogel network, thereby reducing oil loss, while increasing the firmness, cohesiveness, and storage modulus. When model cakes were baked with the oleogels, significant improvement in textural properties was observed with the addition of MAG in the foam-templated oleogels. However, in comparison with shortening-based cakes, oleogel-based cakes still showed a negative effect on hardness, chewiness and cohesiveness.

Keywords : pulse proteins; foam-templated oleogel; monoacylglycerol; candelilla wax; cake baking; texture profile analysis

Introduction

Self-standing solid-like structures from liquid vegetable oil, the so-called oleogels, has recently received significant attention due to its potential to replace trans-fat and reduce saturated fats in confectionery, baked products, and processed meats (Marangoni, Van Duynhoven, Acevedo, Nicholson and Patel, 2020) In conventional oleogels, the structure-forming agents are typically small molecule oleogelators, such as plant waxes, fatty esters, monoacylglycerols, and ethyl cellulose (Patel and Dewettinck, 2016, Singh, Auzanneau and Rogers, 2017). However, with more attention towards 'clean label' and 'healthy' alternatives, the use of food hydrocolloids such as gums, and proteins as oleogelators has recently gained more consideration (Doan, Van de Walle, Dewettinck and Patel, 2015, Gravelle, Blach, Weiss, Barbut and Marangoni, 2017, Gravelle and Marangoni, 2018, Lim, Hwang and Lee, 2017). However, these hydrophilic biopolymers are unable to form a crosslinked network via intermolecular interactions if directly added into the oil; therefore, indirect approaches are required to form a structured oil phase. DeVries and coworkers successfully converted whey protein stabilized hydrogels into oleogels by a step-wise solvent exchange process (de Vries, Hendriks, van der Linden and Scholten, 2015, de Vries, Wesseling, van der Linden and Scholten, 2017). ϰ-Carrageenan stabilized hydrogels were also converted into aerogels using supercritical carbon dioxide and then into oleogels by allowing the aerogel to adsorb liquid oil (Manzocco, Valoppi, Calligaris, Andreatta, Spilimbergo and Nicoli, 2017). Combinations of surface active and non-surface active biopolymers, such as hydroxypropyl methylcellulose (HPMC) or methylcellulose (MC) with xanthan gum (Patel, Cludts, Bin Sintang, Lewille, Lesaffer and Dewettinck, 2014); and soy protein isolate with ϰ-carrageenan (Tavernier, Patel, Van der Meeren and Dewettinck, 2017) were also used to develop emulsion-templated oleogels by removing the water using freeze-drying or vacuum-drying process. Patel and coworkers were the first to use an HPMC foam-templated approach to obtain oleogels (Patel, Schatteman, Lesaffer and Dewettinck, 2013). The freeze-dried HPMC foam was able to hold an oil 98 % of the foam weight. However, one of the most popular biopolymers, pulse

proteins, such as those from pea, lentil and faba bean have not been explored significantly for oleogelation, despite their nutritional and functional quality and higher consumer acceptance.

Our recent studies demonstrated that freeze-dried foams stabilized by combinations of a pea or faba bean protein concentrates with xanthan gum (XG) can be used to structure canola oil (CO) (Mohanan, Tang, Nickerson and Ghosh, 2020). The foams prepared with 5 wt% faba bean protein concentrate (FPC) or pea protein concentrate (PPC) with 0.25 wt% XG at pH 7 and pH 9 were able to hold an oil more than 20 – 30 times of their weight. However, about 30-40% of the added oil was leaked out of the oleogel upon centrifugation (Mohanan, Tang, Nickerson and Ghosh, 2020). Due to the poor oil binding capacity of the protein foam-templated oleogels, their rheological properties were unreliable. Higher oil leakage during rheology measurements of the oleogels led to a significantly higher storage modulus (G') compared to the oleogels with higher oil content (or higher oil binding capacity). Also, the baking quality of the cakes baked using the oleogels was poor due to significantly higher hardness and chewiness compared to a cake baked using conventional high-melting shortening (Mohanan, Tang, Nickerson and Ghosh, 2020). Therefore, the objective of the present study was to improve the oil binding properties of the pulse protein foam-templated oleogels using small quantities of conventional oleogelators, such as high-melting monoacylglycerols (MAG) and candelilla wax (CW). We hypothesized that the oleogelators would act as fillers in the protein network to reduce the oil loss (OL) and improve the rheology of the oleogels and therefore improve the textural properties of the cakes. The overall goal was to see how much of the conventional high-melting shortening functionality can be mimicked by the pulse protein-based oleogels.

Experimental Procedures

Materials

Pea protein concentrate (PPC, PP55) and Faba bean protein concentrate (FPC, FBP60) were kindly donated by AGT Food and Ingredients (Saskatoon, SK, Canada). Proximate analysis of the pulse protein samples is given in Table S1 (Supporting Information). The protein content of PPC and FPC was 51.4 and 57.6 wt%, respectively. Gluten-free xanthan gum (XG) was purchased from Bulk Barn store, a local supplier of Duinkerken Foods Inc. (Slemon Park, PE, Canada). Vegetable shortening (Crisco brand, composed of soybean oil, hydrogenated palm oil, modified palm oil, mono and diglycerides, TBHQ and citric acid) and canola oil (Great Value brand) were purchased from Walmart supercentre (Saskatoon, SK, Canada). Refined candelilla wax was donated by Multiceras (Monterrey, NL, Mexico). Powdered monoacylglycerol (MAG, product code DMG0093) was donated by Palsgaard (Palsgaard Industry de Mexico, San Luis, S.L.P., Mexico). According to the supplier analysis report, the mixture of monoacylglycerols contained about 37% glycerol mono-stearate, 54% glycerol mono-palmitate, and 7.5% free fatty acids with a melting point ~70 ºC. Deionized water (Synergy UV Water Purification System, Millipore Sigma, Oakville, ON, Canada) was used for all the solution preparation. Sodium azide and all other chemicals were purchased from Sigma Aldrich Canada (Oakville, ON, Canada).

Foam preparation

Foams were prepared using a mixture of 5 wt% protein (PPC or FPC) and 0.25 wt% XG at pH 7, according to Mohanan, Nickerson and Ghosh (2020). Protein and XG solutions were prepared separately and stirred overnight at room temperature for proper mixing. Sodium azide (0.02 wt%) was added to the solutions to prevent microbial growth. The required amount of protein and XG solutions were mixed to make up a volume of 400 mL and stirred using a magnetic stirrer (400 rpm) for 30 min. The pH was then adjusted to 7 using 1 M NaOH prior to foam preparation. A KitchenAid Ultra Power Mixer (KitchenAid, Whirlpool Canada, Mississauga ON) with a 4.5 qt (4.3 L) stationary bowl and stainless-steel rotating beaters were used for foam formation. The mixtures of the protein and XG solutions (400 mL) were whipped at speed setting 8 (380 rpm) for 20 min. The foams were immediately transferred to a 20 cm × 20 cm aluminum tray and stored

at -30 ⁰C for 24 h, followed by freeze-drying for 72 h (FreeZone 18 Liter Console Freeze Dryers, Labconco Corp, Kansas City, MO, USA).

Oleogel preparation and characterization

Preparation of oleogel

Oleogels were prepared by adding a hot mixture (80 °C) of CO with 0 – 3% CW or MAG into 0.5 to 1 g of freeze-dried foams taken in 50 mL centrifuge tubes. The lipid mixture was prepared by dissolving the required amount of CW or MAG to CO at 80 ⁰C. The addition of oil mixture was stopped when the foam was saturated with the oil, and the excess oil started coming off when the tubes were inverted. The tubes were then quickly transferred to a refrigerator (4⁰C) to allow the formation of oleogel. The samples were left in the refrigerator for 24 h prior to any measurements. As a control, CO containing different concentrations of CW or MAG (without foam formation) was also added to centrifuge tubes and left in the refrigerator. Foam-templated oleogel with CO without any CW or MAG was also kept at the refrigerator as another control.

Determination of oil loss from oleogels

Oil loss (OL) from the oleogels was used as an indicator of their oil binding capacity (OBC). OL was determined using a method described by () with slight modification. The tubes with oleogels taken out of the refrigerator were centrifuged for 20 min at 300×g using a tabletop centrifuge (IEC clinical centrifuge, Damon Corp., Needham Heights, MA, USA) to remove the excess oil. After centrifugation, the tubes were inverted and placed on a metal wire mesh, which kept on top of a beaker, where the released oil was collected. The weight of the tube before and after removing the released oil was noted. The percent oil loss was calculated using equation 1,

$$\text{Oil Loss} = \frac{W_a - W_b}{\text{Weight of the oil added into the foam}} x \ 100\% (1)$$

where W_a and W_b are the weights of the tube before and after removing the released oil during centrifugation, respectively.

Microstructure of oleogels

The microstructure of the oleogels was obtained using a bright-field polarized light microscope (Nikon Eclipse E400 microscope, with a Nikon DS-Fil camera, Nikon Canada Inc., Mississauga, ON) with a 10× objective lens at room temperature (25 ± 2 ⁰C). A small amount of freshly-prepared molten oleogel mixture was placed on a glass slide, covered with a coverslip, and transferred to a refrigerator for the formation of oleogels. The microscopy of the oleogels was performed at least after 24 h of storage in the refrigerator.

Viscoelasticity of oleogels

Viscoelasticity of the oleogels was measured by a rheometer (Model AR-G2, TA Instruments, Montreal, QC, Canada). A 40mm cross-hatched parallel plate geometry was used for viscoelasticity analysis to eliminate any wall-sleep during measurement. Oleogels were gently loaded on the Peltier plate of the rheometer with a spatula. Excess gel came out after placing the geometry was gently wiped off to maintain the correct level. An oscillatory strain sweep (from 0.01 % to 100 %) was applied at a constant frequency of 1 Hz at 25 ⁰C to find out the linear viscoelastic region (LVR), and then a frequency sweep measurement was performed from 0.01 to 100 rad/s at a constant strain of 0.05 % within the LVR. The storage (G') and loss modulus (G") of the samples was recorded with the TRIOS Software (version 4.0.2.30774, TA Instruments, Montreal, QC, Canada).

4

Spreadability of Oleogel

Spreadability of the oleogels was performed using a TA-425 TTC probe (spreadability-TTC, spreadability RIG) using a texture analyzer (TA-Plus Texture Analyzer, Texture Technologies Crop. Hamilton, MA, USA) at compression mode with a penetration depth of 65 mm, test speed of 3 mm/s and post-test speed of 10 mm/s. Before the measurements, the weight and distance calibrations were performed. The oleogel sample was placed into the female cone, pressed gently to avoid incorporation of air using a plastic spatula, and fixed on the bottom platform of the texture analyzer. The sample surface was levelled, the male cone was placed at a defined position (65 mm) above the sample surface, and the experiment started with the male cone's downward movement (65mm penetration at 3 mm/s speed), which compressed the sample forcing it to flow between the surfaces of the two cones and return to the initial position. From the obtained graphic of force vs. distance, the maximum positive force required for the male cone to penetrate through the sample (firmness) and the maximum negative force (cohesiveness) were calculated.

Preparation and characterization of cakes

Cake baking with oleogel

Cakes were baked using a slightly modified AACC International Method 10-90.01 (AACC, 1999). In each replicate, batters were prepared using 200 g of all-purpose flour, 280 g of crystalline sugar, 100 g of fat, 24 g of non-fat dried milk, 18 g of dried egg white powder, 6 g of NaCl, 12.5 g of baking powder, and 250 g of water. Mixing of the ingredients was done using a KitchenAid Ultra Power Mixer (KitchenAid, Whirlpool Canada LP, Mississauga ON) with a 4.5 qt (4.3 L) stationary bowl and rotating stirrers. For the fat phase of the batter, either vegetable shortening, or CO, or FPC and PPC foam-templated oleogels prepared using 3% MAG and 3% CW were used. As a control, oleogels prepared using 3% CW, 3% MAG in CO, and freeze-dried foams of PPC-XG and FPC-XG in CO were also used. Only the 3% lipid additives were chosen for cake baking because the best properties of the oleogels were obtained at this concentration. All the dry ingredients were mixed before adding them into the mixing bowl. Then the fat phase was added with 150 mL of water and mixed for 1 min at speed 2 followed by 4 min at speed 4. The rest of the water was added in steps with various mixing speeds according to the AACC method. Cakes were baked in an electric oven at 190 °C (375 °F) with about 200 grams of dough placed in a baking tray (15.0 × 7.5 × 5.5 cm). After baking, the cakes were cooled at room temperature for 30 min, carefully removed from the tray, and covered with aluminum foil and plastic wrap to prevent moisture loss until further analysis.

Characterization of cake batters and cakes

The microstructure of the batters was obtained using a light microscope (Nikon Eclipse E400, Nikon Canada Inc., Mississauga, ON) with a 10× objective lens at room temperature. Batters were analyzed within 30 min after preparation to minimize the effect of time. A small amount of batter was taken on a glass slide, compressed gently with a cover slide and used for image capturing.

The viscosity and viscoelasticity of cake batters were measured using the AR-G2 rheometer (TA Instruments, Montreal, QC, Canada) with a 40 mm acrylic parallel plate. Viscosity measurements were done between the two parallel plates at 25 °C with a gap of 500 μm and as a function of increasing shear rate from 0.01 to 1000 s^{-1}. To find out the linear viscoelastic region (LVR), G' and G" were measured at a constant frequency of 1 Hz at 25 °C by controlling the oscillatory strain sweep from 0.01 % to 100 %. Then a frequency sweep measurement was performed from 0.01 to 100 rad/s at a constant strain of 0.05 % within the LVR.

The specific gravity of cake batters was measured as the ratio of the weight of a certain volume of cake batter to the same volume of water. The volume of the cakes was determined using rapeseed displacement procedure according to AACC 10-05 method (AACC, 2001), and the specific volume of the cakes was measured as the cake volume per unit weight of the cake (cm^3/ g).

The texture profile of the cakes was measured by a two-bite test using a texture analyzer (TA-Plus texture analyzer, Stable Micro Systems Ltd. Surrey, UK), according to Kim, Lim, Lee, Hwang and Lee (2017) with

5

slight modification. The measurements were done 24 h after baking using cylindrical pieces (2 cm cubes) of cake crumbs. A cylindrical probe of diameter 2.5 cm, attached to the texture analyzer, was used to compress the cake pieces two times at a speed of 2 mm/s until the height of the cake pieces were half. The texture profiles were analyzed using the Exponent software (version 6.1.4.0, Stable Micro Systems Ltd., Surrey, UK), according to Friedman, Whitney and Szczesniak (1963) to calculate the hardness, springiness, chewiness and cohesiveness of the cakes.

Statistical Analysis

All the measurements used in this manuscript were carried out in triplicate for different foam, oleogel and cake samples, and the results reported are average and standard deviation of these measurements. The results were statistically analyzed from the analysis of variance and t-test at a significance level of 5% using Microsoft Excel 2013.

Results and Discussions

Oleogelation using pulse protein stabilized foams in the presence of CW or MAG

Both FPC-XG and PPC-XG foams readily adsorbed the added canola oil about 30-times their weight with and without CW or MAG and produced a self-standing solid-like structure after 24 h of storage in a refrigerator. No oil came out from any oleogels while inverting the tubes after storing the refrigerator, except the oleogels consists of only less than 1% CW or less than 2% MAG as oleogelator. The critical concentration (the concentration of oleogelator at which the oleogel doesn't flow while inverting the tube) of candelilla wax for gelation of canola oil was previously reported as 1% (Blake, Co and Marangoni, 2014), which matched with the present study. However, no such report on the critical concentration of a similar MAG in canola oil at the same condition was found.

Microstructures of different oleogels (Fig. 1) demonstrated a crystal network of CW and MAG in the foam-templated oleogels. Oleogels stabilized only by CW exhibited network of finely dispersed small grain-like crystals (Fig. 1m), while MAG oleogel displayed a dense network of fibril-like crystals (Fig. 1n). Similar microstructures of CW in CO has been reported before (Blake, Co and Marangoni, 2014). The microstructure of MAG oleogel was similar to the oleogel prepared using safflower oil with 4% MAG (the same ingredient used in the current study) as reported by Lopez-Martinez, Morales-Rueda, Dibildox-Alvarado, Charo-Alonso, Marangoni and Toro-Vazquez (2014), but a denser network and smaller crystals were found in the current study, which might be due to a different cooling rate and the use of different vegetable oil.

In the foam-templated oleogels with and without CW or MAG, dark rings of protein foam boundaries were observed under non-polarized light (Fig. 1d-f, and Fig. 1j-l), and crystal network of CW or MAG, similar to that of CW or MAG-only oleogels, were observed under polarized light (Fig. 1a-c, and Fig. 1g-i). This confirms that the formation of CW or MAG crystal network formed within the foam-templated oleogels without altering any protein foam network. Birefringent starch particles with spherulitic microstructures were also observed in the oleogels stabilized by pulse protein foams with and without CW or MAG under polarized light. More starch granules can be seen in FPC oleogels (Fig. 1g-i) compared to PPC oleogels, as the former had more than double starch content (See Table S1, Supporting Information for proximate analysis of the pulse protein samples).

Oil loss from the oleogels

Oil loss (OL) is an indicator of oil binding capacity (OBC) of oleogel, higher the OL means lower the OBC. The OL of different oleogels is displayed in Fig. 2a and 2b. As expected, the OL of both FPC-XG and PPC-XG foam-templated oleogels significantly reduced with the addition of both CW and MAG, indicating

an increase in OBC of the foam-templated oleogels with these additives. When there was no additive, the OL was about 34.4 +- 5.2 % and 32.4 +- 7.1 % in the case of PPC-XG and FPC-XG foam-templated oleogels, respectively. The OL became zero with more than 2% CW and remained at 4.5 +- 0.2 % and 3.3 +- 0.5 % with 3% MAG in PPC-XG and FPC-XG foam-templated oleogels, respectively (Fig. 2a). It should also be noted that CW or MAG alone cannot bind oil as good as in combination with a protein stabilized foam. The oleogels made only with 1% or less than 1% CW or MAG displayed 100% OL (Fig. 2b), showing complete disruption of crystal network made of CW or MAG under centrifugation. No oil was released when 2 % or more CW was present in CO; however, about 7.0 +- 0.6 % oil was released even when 3 % MAG was present in the CO. Previously, a significantly higher oil loss was reported in MAG-stabilized canola oil oleogels, where even with 8% MAG about 50 % of the oil was lost from the oleogel during storage on a filter paper for 15 days (Lopez-Martinez, Charo-Alonso, Marangoni and Toro-Vazquez, 2015). This difference could be attributed to the use of different methods and timescale to measure the OL. Lower OL (higher OBC) of oleogels containing CW indicates that the crystal network of CW plays a critical role in determining the OL. Blake, Co and Marangoni (2014) showed that lower OL could be obtained if the oleogelator can cover more surface area in the oleogel. Although the dense crystalline network was found in both CW and MAG systems (Fig. 1), higher OL in MAG oleogels compared to CW oleogels indicates that needle-shaped MAG crystals (Fig. 1n) formed a weaker network compared to granular and spherulitic CW crystals (Fig. 1m) in binding CO and preventing disruption during centrifugation. Although most of the oil was released from the CW or MAG-only oleogels during centrifugation (with 1% or below oleogelator), the presence of protein foam alone significantly reduced OL (Fig. 2a), which was further improved when the oleogels were stabilized by protein foam along with CW or MAG.

Intermolecular interactions such as hydrogen bonding, hydrophobic interactions and van der Waals interactions could be the factors that helped both CW and MAG crystal network and the foam network to bind canola oil. In a previous study, it was demonstrated that major interaction that helped the protein foam network to bind canola oil was hydrophobic and van der Waals interactions, evidenced by changes in protein secondary structure and changes in corresponding interaction bands in FT-IR spectra (Mohanan, 2020). Weak intermolecular hydrogen bonding was detected in MAG-stabilized oleogels (Lupi, Greco, Baldino, de Cindio, Fischer and Gabriele, 2016, Ogutcu and Yilmaz, 2014) as well as in natural wax stabilized oleogels (). The strong crystalline network formed through intermolecular interactions might have trapped the liquid oil presented in the protein foam stabilized oleogels, thereby further reinforcing the overall oleogel network and reducing OL.

Oleogel viscoelasticity

Rheological properties of the oleogels are important to understand their gel strength and better define their application. Oscillatory strain-dependent viscoelastic measurements of different oleogels were performed at a constant frequency of 1 Hz to find the linear viscoelastic region (Fig. S1, Supporting Information). At low strain %, the storage moduli (G') of all samples, except 0.5% CW, and 0.5% MAG, were higher than that of loss moduli (G"), indicating an elastic behaviour. An LVR was observed until about 0.1% strain. With an increase in strain, G' decreased, indicating yielding, followed by a cross over between G' and G", representing a loss of gel structure.

Frequency-dependent viscoelastic measurements of all oleogels were performed within the LVR and are shown in Fig. S2 (Supporting Information). Storage moduli (G') of all samples, except as that of 0.5% CW, and 0.5% MAG, were higher than loss moduli (G"), indicating an elastic behaviour of the gels at the performed range. All oleogels with CW (Fig. S2a, c, e) displayed similar changes in G' and G" with frequency, G' increased linearly with frequency. In contrast, all oleogels with MAG (Fig. S2b, d, f) displayed a slightly higher rate of increase in G' at a lower strain, which decreased, making G' more independent at a higher frequency. Overall, the slope of the G' curve was only slightly positive for both CW and MAG, indicating limited rearrangement of the gel structure at a shorter time scale (or higher frequency). For all oleogels, G" decreased first to a minimum and then increased with frequency (Fig. S2a, c, e), and this behaviour was

7

evident in oleogels with more than 1% CW or MAG. The minima in G" as a function of frequency indicates motion in the gel network from a more rigid in-cage structure to a more fluid out-of-cage structure at a shorter time scale (higher frequency) and weakening of the gel structure (Zhou, Hollingsworth, Hong, Cheng and Han, 2014). Interestingly, with an increase in concentration, the minimum values of G" shifted to a higher frequency in case of the MAG-based oleogels (Fig. S2b, d, f), while it shifted to lower frequencies in case of CW-based oleogels (Fig. S2a, c, e). Similar rheological behaviour was observed for oleogels of vegetable oil stabilized by sorbitan and glycerol monostearate and is common for network-based gelled dispersions (Sanchez, Franco, Delgado, Valencia and Gallegos, 2011).

To compare the gel strength of different oleogels, G' and G" of different oleogels at 10 rad/s were replotted in Fig. 3. Both G' and G" increased with an increase in CW or MAG content, except for FPC foam-templated oleogels (Fig. 3b), where no change was observed at 2 and 3% CW or MAG concentrations. Although the viscoelastic moduli values were much lower for the oleogels without foam, the rate of increase as a function of CW and MAG concentration was much higher (Fig. 3c) compared to the foam-templated oleogels. The increase in viscoelastic moduli could be due to the increase in solid content and the strength of crystal network with an increase in the concentration of CW or MAG. With the presence of a protein foam network in the oleogels, the effect of CW or MAG concentration on gel strength was less visible, especially at 2 and 3% concentrations. There were no significant differences between the G' and G" values when PPC-XG was replaced by FPC-XG (p>0.05) in foam-templated oleogels at all CW or MAG concentrations (Fig. 3a, b). In the absence of foam, the oleogels containing CW displayed higher G' and G" values than the oleogels made of MAG at all concentrations (Fig. 3c), which indicates that the CW crystal network was stronger than that of MAG and are less prone to deformation. The order of magnitudes of G' and G" of 3 % MAG oleogel was close to that of 4 % MAG oleogel created with safflower oil (Lopez-Martinez, Charo-Alonso, Marangoni and Toro-Vazquez, 2015). Surprisingly, the foam-templated oleogels containing MAG displayed higher G' and G" than the corresponding CW containing oleogels at all concentrations (Fig. 3a, b). This might be due to the higher oil loss of MAG containing oleogels (Fig. 2). In our previous study, it was also demonstrated that when the oil content was lower, the foam-templated oleogels displayed higher G' and G" due to the stronger effect of the protein network on oil gelation (Mohanan, Tang, Nickerson and Ghosh, 2020).

Oleogel spreadability

The firmness and cohesiveness of all oleogels were measured using the spreadability test and are shown in Fig. 4. Firmness and cohesiveness indicate the easiness of spreading and handling of oleogels; the higher the firmness, the harder it is to spread the oleogel (lower spreadability), and higher the cohesiveness, the stickier the oleogel. As shown in Fig. 4, both the firmness and cohesiveness of all oleogels increased with an increase in CW and MAG concentrations as expected. When there was no foam present (Fig. 4c and 4d), The firmness and cohesiveness of the oleogels were much lower compared to the foam-templated oleogels (Fig. 4a and 4b). Between these two oleogels without foam, CW oleogels displayed higher firmness and cohesiveness than MAG oleogels (Fig. 4c and 4d). This agrees with the rheology results, where the G' of CW oleogels was significantly higher than that of MAG oleogels. Therefore, it is reasonable to assume that the firmness of oleogels is associated with the strength of the crystal network and its ability to retain oil. In both FPC-XG and PPC-XG foam-templated oleogels, however, there was no significant difference in firmness or cohesiveness when CW was replaced by MAG (p>0.05) at all concentrations, except when the cohesiveness decreased at 3% oleogelator concentration. Nonetheless, unlike what has been observed in rheological properties, FPC-XG foam-templated oleogels displayed higher firmness and cohesiveness than corresponding PPC-XG foam-templated oleogels with and without CW or MAG.

Cake baking with oleogel

Conventionally, a highly saturated fat containing shortening is used in cake baking to stabilize air bubbles in the batter and to provide the right rheology of the batter to give a soft tendered final cake product (Wilderjans, Luyts, Brijs and Delcour, 2013). In the present work, shortening was fully replaced with

8

oleogels to determine how much of these oleogels can mimic the functionality of shortening. To do this, we used PPC-XG and FPC-XG foam-templated oleogels with and without 3% CW and 3% MAG, and oleogels made of 3% MAG and 3% CW alone. As a control, the cake was also baked using 100% CO as a control. Note that the oleogels used in cake baking contained more than 95% CO.

Properties of cake batters

The specific gravities of the cake batters prepared using vegetable shortening, CO and the various oleogels are shown in Table 1, and their microstructures obtained using a light microscope are shown in Fig. 5. The batter prepared using shortening displayed the least specific gravity, indicating higher incorporation of air into the batter (Wilderjans, Luyts, Brijs and Delcour, 2013). This is also evident in their microscopic image, where numerous small air bubbles can be observed (Fig. 5). The conventional shortening used in the research consists of solid fat crystals and mono and diglyceride emulsifiers, which are capable of stabilizing air bubbles in the batter (Wootton, Howard, Martin, McOsker and Holme, 1967). Batter prepared using CO displayed the highest specific gravity, which could be due to the inability of the liquid oil to stabilize air bubbles in the batter. As illustrated in Fig. 5, there were only a few air bubbles incorporated in CO batter. Oleogels consist of only foams or CW or CW with foam displayed similar but significantly higher specific gravity compared to shortening. The microscopic images of these batters shown in Fig. 5 also confirmed the incorporation of fewer air bubbles in the compared to shortening batter. Among all the oleogels, those consist of MAG with or without foam displayed lowest specific gravity; however, they were still higher than that of shortening batter (Table 1). The oleogels consist of MAG and protein foams displayed similar specific gravity as that of batters with only MAG oleogel (p > 0.05), indicating the presence of the protein foams did not help further air incorporation in the batter. Microscopic images of batters containing MAG clearly showed the incorporation of a greater number of air bubbles in the batter compared to CO, only protein foams and CW batters (Fig. 5). It also shows that the presence of solid fat crystals made of MAG was crucial for the stabilization of air in the batter, which could be ascribed to the surface activity of the MAG. Although CW was able to form crystals in CO, the cake batters prepared using only CW displayed almost similar specific gravity as that of CO batter (p > 0.05). The addition of protein foams in the oleogels with CW decreased the specific gravity, but it not significantly different than the batters prepared using only protein foam-templated oleogels. These results show that not only improvement on gel strength or OBC of oleogels can improve the quality of batters, but the ability of the oleogelators to increase the stabilization of air bubbles does matter.

It is known that the rheological properties of the batters determine the functional properties of the final product (Ronda, Oliete, Gomez, Caballero and Pando, 2011). Therefore, both flow and viscoelastic behaviour of all batters were determined (Fig. 6). All cake batters displayed pseudoplastic behaviour, where viscosity decreased with an increase in shear rate (Fig. 6a). The flow curves of all cake batters were fitted to a power-law model ($n = K\dot{\gamma}^{n-1}$), where n is the viscosity, ($\dot{\gamma}$) is the shear rate, K is the consistency coefficient, and n is the flow behaviour index. The fitting parameters are shown in Table 2 (R^2 values were > 0.83 for all). Flow behaviour indices of all batters were less than 1, and shortening batter displayed the lowest flow behaviour index followed by the oleogel batters consisting of both foam and MAG, indicating more shear thinning and the formation of higher-order structure. Batters with both foam and CW displayed the highest 'n' values (Table 2), indicating less shear thinning behaviour. Moreover, the shortening batter displayed the highest consistency index (K), indicating higher viscosity at 1 s⁻¹ shear rate than that of all other batters (Fig. 6a). The higher low-shear viscosity of shortening batter might be attributed to solid fat crystals and higher air incorporation. At higher shear rates, the viscosities of all batters were close to each other, where the fat crystal network present in the shortening could be disrupted and the air bubbles collapsed, leading to a reduction in viscosity. Due to the inclusion of a higher portion of liquid oil in the oleogel and CO batters, they displayed lower viscosities than the shortening batter. Reduction in viscosity while replacing shortening with rapeseed oil was previously reported (Hesso, Garnier, Loisel, Chevallier, Bouchet and Le-Bail, 2015). The n and K values of the PPC and FPC foam-templated oleogel cake batters were also comparable to that of muffin batters prepared using HPMC foam-templated oleogel batters (Lee, 2018), indicating similar

functionality of protein and HPMC foam in oleogels.

All batters displayed tan δ (ratio of G" to G') values lower than 1 (Fig. 6b) and higher G' than G" at the whole frequency range studied (Fig. 6c and 6d), indicating elastic behaviour. Tan δ for shortening batter decreased with an increase in frequency, indicating dominant elastic behaviour. All batters displayed similar tan δ to the shortening batter below 1 rad/s frequency, however, it raised beyond 1 rad/s and exceeded the value of shortening batter, indicating replacement of shortening with oleogels led to more contribution to the viscous nature of the batter. Both G' and G" also increased linearly with increase in frequency, indicating structural changes with the lowering of the timescale of the applied strain. The increase in G' with frequency was less in shortening batters compared to the others. When shortening was replaced with oleogels or CO, both G' and G" reduced significantly. The batters prepared using wax-based oleogels (Lim et al., 2017, Kim et al., 2017) and HPMC foam-templated oleogel (Lee, 2018) also displayed similar viscoelastic behaviour as that of the batters used in the current study. Both G' and G" increased with increase in frequency when shortening was replaced with HPMC foam-templated oleogels (Lee, 2018). Lower viscosity and viscoelastic values of oleogel batters compared to shortening batter could be related to partial replacement of solid fat by liquid oil and lower air incorporation. An increase in specific gravity due to lower air incorporation was led to a reduction in cake volume and an increase in cake hardness (Sahin, 2008). The ability of the batter to retain the air determines their rheological properties and the quality of the final product.

Properties of cakes

The cross-sectional view of the final cake products and their specific volumes (cm^3/g) are provided in Fig. 7 and Table 1, respectively. Shortening cakes displayed the uniform distribution of open air cells and granular particles of flour solids, while cakes prepared using CO and oleogel displayed the relatively non-uniform distribution of large air bubbles dispersed in a dense network of cake matrix. Oleogels prepared using both the foams with and without CW or MAG displayed similar cakes appearance. However, contradictory to what has been observed in the literature (Sowmya, Jeyarani, Jyotsna and Indrani, 2009), there was no volume reduction observed when shortening was replaced by liquid oil or oleogel, all cakes displayed similar specific volume (p>0.05). These results confirm that the rheology or specific gravity of the batters cannot be used to predict the final cake volume. There might be interactions with other ingredients during baking, which determined the volume of the final product.

Textural properties of cakes (hardness, springiness, cohesiveness, and chewiness) are displayed in Fig. 8. Hardness is an indicator of staling of baked products and can be determined using the force required to compress the sample to a certain height. The shortening cakes displayed the lowest hardness (p < 0.05), followed by the hardness of cakes prepared by PPC and FPC foam-templated oleogels containing MAG, which were lowest among all the oleogel and CO-added cakes (Fig. 8a). Cakes prepared using CO and CW oleogel displayed similar, but highest hardness among all the cakes where shortening was replaced with another lipid. All other oleogel cakes (MAG, PPC-XG, FPC-XG, PPC-XG+CW, and FPC-XG-CW) displayed similar hardness. The hardness of the cakes correlated well with their batter specific gravity. Batters with lower specific gravity displayed lower hardness, and these results are supported by the literature (Sahin, 2008). Higher hardness also indicates higher protein-protein and protein-gelatinized starch interaction (Paraskevopoulou and Kiosseoglou, 1997). The monoacylglycerols/diacylglycerols present in the shortening interacted with starch and reduced its interaction with protein and hence lowering of the staling process (Cauvain, 1998, Mattil, 1964), which led to the lowest hardness of shortening cakes and lower hardness of MAG-based oleogel cakes. In addition to this, shortening saturated fat crystals' ability to prevent the formation of gluten network might have also led to lower hardness. Generally, springiness values (Fig. 8b) were found to be very close to each other, although CO cake displayed slightly higher, and MAG oleogel cake displayed slightly lower springiness than shortening cake (p < 0.05). Springiness is an indication of the elasticity of cake, and our results showed that all the cakes had almost similar capacity to recover its deformation after exposed to any external force. Cohesiveness is a direct function of work required to breakdown the internal bonds between different ingredients in the cake matrix during each chew or the internal resistance of food to traction, which was determined from the ratio of the second to the first peak area of the

two-bite test. Shortening cakes displayed the highest cohesiveness followed by all CO and all oleogels cakes, except the cakes made of oleogels consist of CW, which displayed the lowest cohesiveness (Fig. 8c). This indicates that the replacement of shortening with liquid oil and oleogels make it easy to disintegrate the cake matrix during mastication. Both springiness and cohesiveness did not correlate well with any batter characteristics studied, such as viscosity, viscoelasticity or specific gravity. Therefore, springiness and cohesiveness of cakes could be influenced by the changes in the interaction between the ingredients and how they form the structure in the cake matrix during baking. The chewiness of the cakes followed a similar trend as that of hardness, except the CW oleogel cake, which displayed lower chewiness than CO cakes (Fig. 8d). Chewiness indicates the energy required for mastication, which was calculated from the product of primary parameters hardness, cohesiveness and elasticity (Friedman, Whitney and Szczesniak, 1963). Chewiness values indicate that the CO cakes required the highest energy to masticate, and the replacement of CO with all oleogels decreased the chewiness to a level similar to shortening-based cake.

Conclusions

The effect of small fractions of high melting candelilla wax (CW) and monoacylglycerols (MAG) in pea (PPC) or faba bean protein concentrate (FPC) plus xanthan gum (XG) foam-templated oleogels on oil loss, rheological properties, spreadability and functionality as a shortening alternative in cakes were examined. The study showed that the addition of 3% CW and MAG in foam-templated oleogels could prevent or significantly reduce oil loss, and increase storage and loss modulus ˜ 1000 times, and firmness and cohesiveness by ˜ 3 times. Although CW was more effective in reducing the OL, and increasing the rheological properties, MAG was more effective in improving the functional properties of cake batters and cakes. Even though it was not as good as shortening baked cakes in terms of hardness, cohesiveness and chewiness, hardness and chewiness of foam-templated oleogel cakes were significantly reduced with the incorporation of MAG into the oleogel, rather than using liquid canola oil.

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