

Effects of storage temperature and duration on physical and microstructure properties of superolein oleogels

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SUMMARY: The effect of storage temperature and duration on the stability of oleogels produced from sunflower wax, polyglycerol behenic acid ester, and fully hydrogenated palm-based monoacylglycerols in relation to their physical and microstructure properties was investigated. The oleogel kept at 5 °C exhibited the highest hardness due to the formation of more crystals. Polyglycerol behenic acid ester oleogels showed phase separation and amorphous peaks at high temperatures, indicating instability and lack of crystalline structure. sunflower wax oleogels remained stable and exhibited β' polymorphs with needle-like crystal structures. The palm-based monoacylglycerol oleogels displayed a complex pattern of crystalline and amorphous behavior, with large crystals and voids, resulting in lower stability despite their high hardness. The findings from microscopy and XRD observations highlight the critical role of a gelator type in determining the properties and stability of oleogels. This knowledge enhances our understanding of the behavior of superolein oleogels, which is essential for their application in various industries.

KEYWORDS: Monoacylglycerol; Polyglycerol ester; Sunflower wax; Superolein oleogel.

RESUMEN: *Efectos de la temperatura y el tiempo de almacenamiento sobre las propiedades físicas y microestructurales de los oleogeles de superoleína.* Se estudió el efecto de la temperatura y el tiempo de almacenamiento en la estabilidad de oleogeles producidos con cera de girasol, éster de ácido behénico de poliglicerol y monoacilglicéridos hidrogenados de palma, evaluando sus propiedades físicas y microestructurales. El oleogel almacenado a 5 °C mostró mayor dureza debido a la formación de más cristales. Los oleogeles de ácido behénico de poliglicerol presentaron separación de fases y picos amorfos a altas temperaturas, lo que indica inestabilidad. Los oleogeles de cera de girasol fueron los más estables, con polimorfos β' y cristales en forma de aguja. Los oleogeles de monoacilglicéridos mostraron grandes cristales y vacíos, con menor estabilidad pese a su dureza. Los análisis de microscopía y difracción de rayos X subrayan que el tipo de gelificante es clave para las propiedades y estabilidad de los oleogeles, aportando información útil para aplicaciones industriales.

PALABRAS CLAVE: Cera de girasol; Éster de poliglicerol; Monoacilglicérido oleogel de superoleína.

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1. INTRODUCTION

Oils and fats are vital components in food applications, and their physical properties are influenced by the chemical composition of the fatty acids attached to the glycerol backbone. Traditionally, solid fats were structured using *trans* fat and saturated fats, which provided functionality such as shortening power,

plasticity and elasticity (Edmund and Marangoni, 2012) but they are known to have some negative health implications (Tavernier *et al.*, 2017).

Reducing the presence of *trans* fat and saturated fatty acids poses a significant challenge for food manufacturers due to the vital role these fatty acids play in structuring fats to enhance the functionality of food products (Co and Marangoni, 2012). One potential

solution that has gained attention is organogelation. This technique involves creating a network structure using self-assembled gelator molecules, resulting in low-saturation solid fat products by incorporating minimal amounts of saturated components into high amounts of liquid oil components. Organogelation involves soft matter systems with liquid oil as the continuous phase, entrapped within a three-dimensional network structure formed by self-assembled gelator molecules. Researchers have explored the use of oleogels in various food products. To date, most research on oleogel preparations has focused on oleogel preparation using soft vegetable oils such as canola oil, soybean oil, sunflower oil, safflower oil, rice bran oil and olive oil (Doan *et al.*, 2015; Jang *et al.*, 2015; Toro-Vazquez *et al.*, 2007). However, these oils exhibit certain limitations concerning stability and physical properties, particularly when used in food processing that requires high processing temperatures. Furthermore, an overconsumption of polyunsaturated fatty acids (PUFA) may be detrimental to antioxidant compromised individuals, despite the known beneficial effects in reducing the risk of cardiovascular disease when replacing saturated fat (Sacks *et al.*, 2017). In this context, palm olein, with its balanced fatty acid composition and high stability, presents itself as a promising option.

The unique composition of palm olein, with its relatively higher saturated content compared to other vegetable oils, may play a distinct role in organogelation. However, there is limited literature available on the use of palm-based products such as palm oil, palm olein, super olein, or top olein for oleogel preparation (Baran *et al.*, 2014; Pradhan *et al.*, 2014). Therefore, this study seeks to fill this research gap and explore the possibility of using palm-based oleins as materials for oleogel production. By replacing highly unsaturated, soft vegetable oils with palm-based oleins, it is expected that the chemical and physical stability of the resulting oleogels will improve, rendering them suitable for various food products.

The formation of oleogels is highly dependent on several factors, including temperature, molecular weight of gelators, structure of the solvent (liquid oil), presence of surfactant, concentration, and incorporation method (Zetzl and Marangoni, 2011). This study focused on evaluating the impact of storage temperature and duration when using different

gelators: polyglycerol behenic acid ester (PBA), sunflower wax (SFW) and fully hydrogenated palm-based monoacylglycerols (MGHO). Among these options, SFW has demonstrated significant potential for gelling various vegetable oils (Blake *et al.*, 2014; Hwang *et al.*, 2015; Patel *et al.*, 2015). Monoglyceride (MG) has also been investigated as a gelling material in oil or oil-in-water systems (Bin Sintang *et al.*, 2017; Da Pieve *et al.*, 2011).

In a previous study, the influence of storage temperature and duration on the thermal and rheological properties of superolein oleogels were examined using SFW, PBA and MGHO (Saw *et al.*, 2023). Each gelator showed a distinct performance in gelling the superolein liquid oil, resulting in complicated observations in their rheological and melting properties. The effectiveness of gelators on gelling the superolein oil is mainly dependent on their solubility in the liquid oil, where good gelators will show moderate solubility in the liquid oil (Blake *et al.*, 2014). In addition to their influence on the rheological and thermal behaviours, their effects on the physical properties and microstructure behavior of oleogels are also crucial in determining their overall performance in structuring the superolein oil, because these properties may affect the stability of the oleogels. Therefore, the objective of this research is to investigate the influence of storage temperature and duration on the stability of oleogels in relation to their physical properties and microstructure behaviour.

2. MATERIALS AND METHODS

2.1. Materials

Refined, bleached, and deodorized superolein (POoIV64) was obtained from PGEO Sdn. Bhd. in Johor, Malaysia. The PBA gelator was obtained from Sakamoto Yakuhin Kogyo Co., Ltd., Osaka, Japan. It is a permitted food additive in Japan, which is commercially known as CV-1L, and contains fatty acids which comprise a mixture of stearic acid, oleic acid and behenic acid (Saw *et al.*, 2023). Food-grade SFW with a melting point of 77 °C was obtained from Shri Balaji Driers in Karnataka, India. Fully hydrogenated palm-based monoacylglycerol (MGHO), consisting of 40.9% palmitic acid and 56.8% stearic acid, was sourced from Ecolex Sdn. Bhd., Selangor, Malaysia.

2.2. Composition of palm superolein

Fatty acid composition was analysed in following with the boron trifluoride method according to ISO 12966-2:2011, using a 430-GC gas chromatography (Bruker, Germany) (Saw *et al.*, 2023). Triacylglycerol composition was analyzed using the AOCS Official Methods Ce 5c-89 and Ce 5c-93 (AOCS, 2017a; AOCS, 2017b) (Saw *et al.*, 2023).

2.3. Preparation of superolein oleogels

Oleogels were made following a preliminary investigation which determined that the best gelator concentrations were 3% w/w for PBA and SFW and 8% for MGHO (Saw *et al.*, 2020). Oleogels were prepared in 200-g batches by heating the mixture to 90 °C in an oven for one hour to fully dissolve the gelator. The mixtures were put into their respective containers for various analyses.

2.4. Determination of solid fat content (SFC) using pulsed-NMR

SFC was determined using a minispec mq20 pulsed-NMR from Bruker, Germany. The NMR tubes, measuring 10.0 mm in diameter, 1.0 mm in thickness, and 180 mm in height, were filled with 3 cm melted samples in height. After being left at room temperature for an hour, the tubes were transferred to incubators, set at temperatures of 5, 15 and 25 °C. SFC was measured on days 1, 2 and 3, with day 0 being the day of preparation.

2.5. Measurement of hardness by texture analyzer

The molten gel was placed in polypropylene containers with a 5.5 cm internal diameter and 3.5 cm height in incubators at the storage temperatures. Gel strength was measured using a texture analyzer (TA.XT plus, UK) equipped with a 500-g load cell. The hardness of the oleogel was measured using a cylindrical probe (2 mm diameter) at 1 mm/s and a maximum penetration depth of 8 mm. This method was modified from (Bot *et al.*, 2009).

2.6. Stability of superolein oleogels

The stabilities of the oleogels were analyzed using a centrifugal stability analyzer, LUMiFuge

Model LF111 (LUM Ltd., Berlin, Germany). The sample was placed in a LUMiFuge tube ((PC10 mm with a PP stopper) and stored at room temperature for an hour before being transferred to incubators at 5, 15, and 25 °C. The equipment was preconditioned to the storage temperature before loading the cells into the centrifuge. The spinning speed was set to 4000 rpm for 4 hours, under the respective storage conditions. The analyses were conducted on days 1, 2, and 3. The system continuously recorded near-infrared transmission profile during the process. The data was integrated using LUMiFuge® SEP Vie 5.1 software. The percentage of oil-released was calculated by comparing the oil-released height after the centrifugation to the initial sample height.

2.7. Analysis of crystal polymorphism using X-ray diffractometer

A Rigaku TTRAX III X-ray diffractometer (XRD) (Rigaku, Japan) was used to measure the polymorphism of the oleogel. The oleogel was melted at 90 °C to destroy the crystals' history. The melted oleogel was placed into the XRD sample pans and stored in an incubator for a day. The samples were analyzed at 1 to 30° 2 θ using a Cu source-X-ray tube at 40 kV and 200 mA at 0.5 °/min.

2.8. Morphological study by microscope

The morphology of the oleogels was studied using a Leica DM4500 P LED polarized light microscope (Germany). The microscope was equipped with a LINKAM LTS120 temperature controller, a LINKAM T95 system controller and a JULABO F250 recirculating cooler. The oleogel sample was placed onto a glass slide and pressed with a glass cover slip to ensure thin layer of well-distributed sample. The microscope images were captured at magnifications of 10, 20 and 50x in a standard bright field configuration.

2.9. Statistical analysis

Analysis of variance using Minitab 16.2 was employed to investigate the effect preparation day on oil released, SFC and hardness of the oleogels.

3. RESULTS AND DISCUSSION

3.1. Effects of storage temperature and duration on solid fat content (SFC)

Figure 1 shows the SFC of oleogels from PBA, SFW and MGHO gelators at 5, 15 and 25 °C from day 1 to day 3. As expected, the SFC increased significantly with decreasing storage temperature due to the increase in crystal formation at lower temperature. An extremely high percentage of SFC (55%) was observed at 5 °C for all oleogels on day 2 and day 3, which initiated with much lower SFC. Table 1 shows that superolein contains high-melting triacylglycerols, such as POP and SOS, with a total

TABLE 1. Fatty acid composition and triacylglycerol composition of palm olein of IV64

Composition	
Fatty acids (area %)	
Lauric (C12:0)	0.23 ± 0.00
Myristic (C14:0)	1.12 ± 0.01
Palmitic (C16:0)	35.11 ± 0.08
Stearic (C18:0)	3.57 ± 0.01
Arachidic (C20:0)	0.32 ± 0.01
Palmitoleic (C16:1)	0.22 ± 0.00
Oleic (C18:1)	46.84 ± 0.08
Linoleic (C18:2)	12.34 ± 0.01
Linolenic (C18:3)	0.25 ± 0.01
Saturated (SFA)	40.35 ± 0.10
Monounsaturated (MUFA)	47.06 ± 0.08
Polyunsaturated (PUFA)	12.59 ± 0.01
Triacylglycerols (area %)	
OLL	0.67 ± 0.01
PLL	2.97 ± 0.05
MLP	0.57 ± 0.05
OLO	2.32 ± 0.03
PLO	12.81 ± 0.10
PLP	11.73 ± 0.01
OOO	6.38 ± 0.04
POO	36.59 ± 0.02
POP	19.11 ± 0.12
SOO	3.81 ± 0.06
POS	2.73 ± 0.06
SOS	0.31 ± 0.03

NOTE: Data represent the mean ± standard deviation from duplicate analyses

saturation content of 40.35%. This finding suggests that these high-melting triacylglycerols in superolein crystallized and reached equilibrium at an SFC of approximately 55%.

At 15 °C, PBA oleogels showed the lowest SFC (2.5%) during the 3-days storage. SFW oleogels demonstrated similar SFC on day 1 as the PBA oleogels. However, the SFC was gradually increased from 1.93 to 5.22 and 6.77% on days 2 and 3, respectively. This result aligns with previous findings that

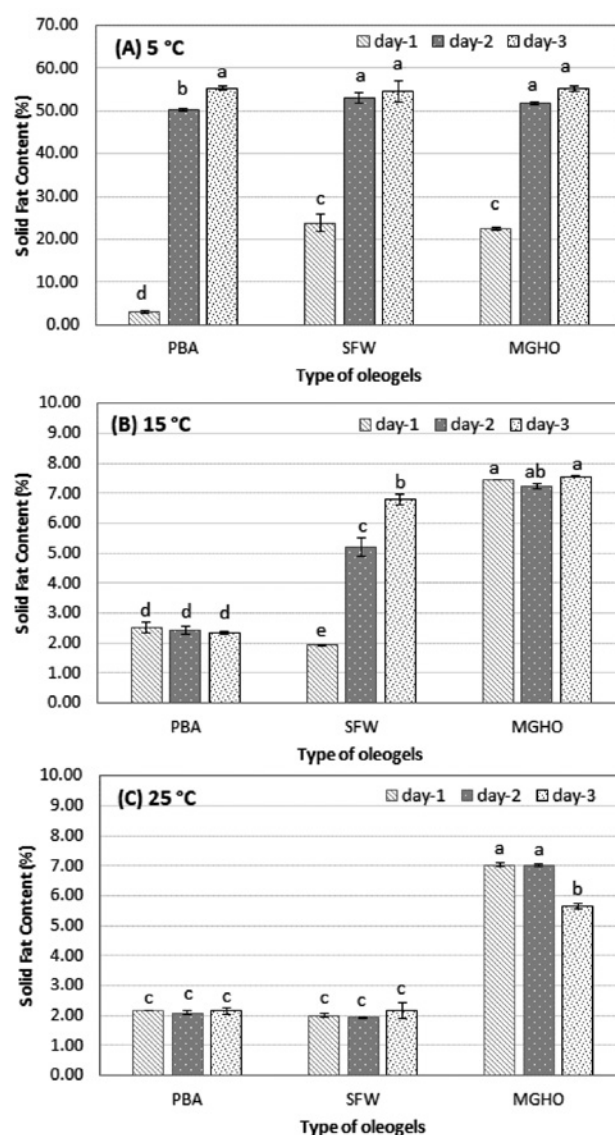


FIGURE 1. Solid fat content of oleogels from PBA, SFW and MGHO gelators at (A) 5 °C, (B) 15 °C and (C) 25 °C on days 1 to 3. Each value represents the mean ± standard deviation of triplicate analyses. Significance testing was conducted between the same kinds of oleogels prepared on different days of preparation.

Different letters indicate significant differences at $p < 0.05$ according to the Tukey's test.

indicated the slow crystallization of superolein at 15 °C storage with SFW; whereas PBA showed crystallization inhibition (Saw *et al.*, 2023).

In contrast, a stagnant SFC (7.5%) trend was observed in MGHO oleogels stored at 15 °C, indicating a similar amount of crystals present during the 3-day storage. This result did not show the same trend as the results previously reported in Saw *et al.* (2023), which showed a drastic increase in G'_{LVR} and G''_{LVR} during the 3-day storage period. However, the thermal analysis results could explain these phenomena, as the melting properties indicated a possible crystal rearrangement during storage, which did not influence the total amount of crystals, thus showing a constant SFC (Saw *et al.*, 2023). Omar *et al.* (2015) reported similar observations, in which RBD palm oil clusters became more spherulitic with increasing size but SFC still remained the same throughout a 1-week storage period. The crystal rearrangement was due to the slow crystallization behavior of palm oil products, providing similar effect to post-hardening.

At 25 °C, PBA and SFW oleogels showed much lower SFC (2%) than the MGHO oleogels (7%), indicating the presence of more crystals in the MGHO oleogel. At 25 °C, some high melting TAGs in the superolein interact with the MGHO molecules to form more crystals, thus leading to a higher SFC in MGHO oleogels. Interestingly, MGHO oleogels showed very similar SFC when stored at 15 and 25 °C, meaning that the difference in the storage temperature did not alter the total amount of crystals formed. More analyses are required to explain this observation.

3.2. Effect of storage temperature and duration on hardness

The hardness levels of superolein oleogels are illustrated in Figure 2. The oleogels at 5 °C showed the highest hardness, ranging from 310 to 440 g, but the hardness reduced very drastically in stages to approximately 1.5-23 g at 15 °C and to 0.8-1.6 g at 25 °C. At 15 °C, PBA oleogels showed a significantly lower hardness of 1.5 g, whereas SFW and MGHO oleogels showed relatively higher hardness levels during the 3-day storage period. A drastic increases in hardness were observed in SFW and MGHO oleogels during the 3 days of storage, which was in line with the rheological results (Saw *et al.*, 2023). This was also due to the slow crystallization behavior of superolein at 15 °C.

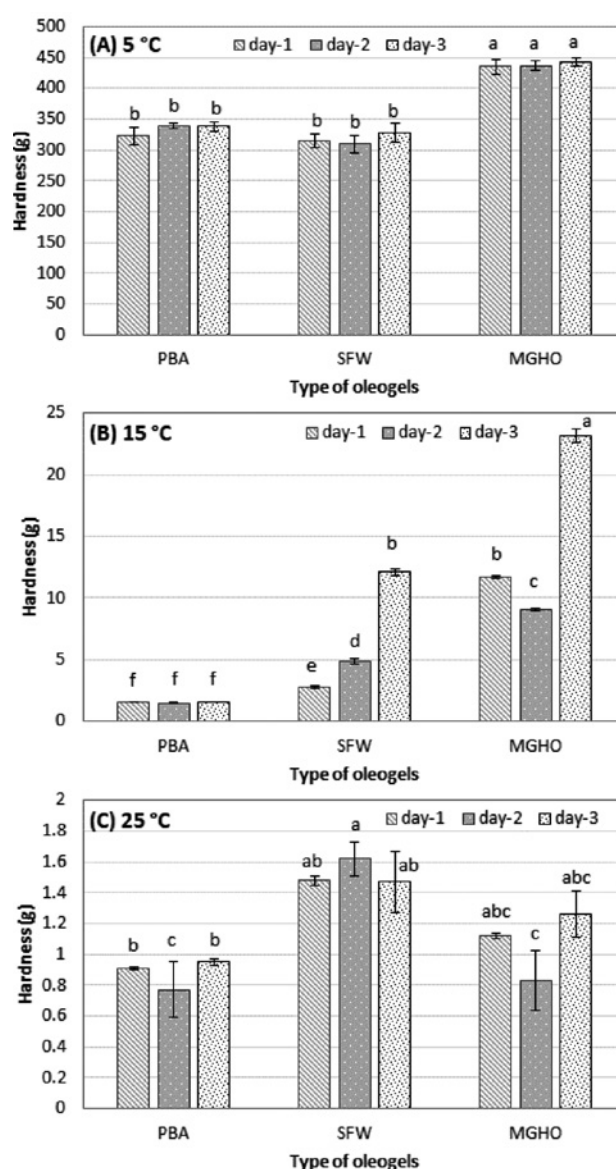


FIGURE 2. Hardness of oleogels from PBA, SFW and MGHO gelators at (A) 5 °C, (B) 15 °C and (C) 25 °C on days 1 to 3. Each value represents the mean $\pm$ standard deviation of triplicate analyses. Significance testing was conducted between the same kinds of oleogels prepared on different days of preparation. Different letters indicate significant differences at $p < 0.05$ according to the Tukey's test.

3.3. Effect of storage temperature and duration on polymorphism

Figure 3 shows the XRD diffractograms of the superolein oleogels on day 1. In both the SAXS and WAXS region, PBA oleogels did not show clear crystalline peaks for oleogels stored at 15 or 25 °C. This finding indicated that no crystalline particles were involved in the structuring of PBA oleogels,

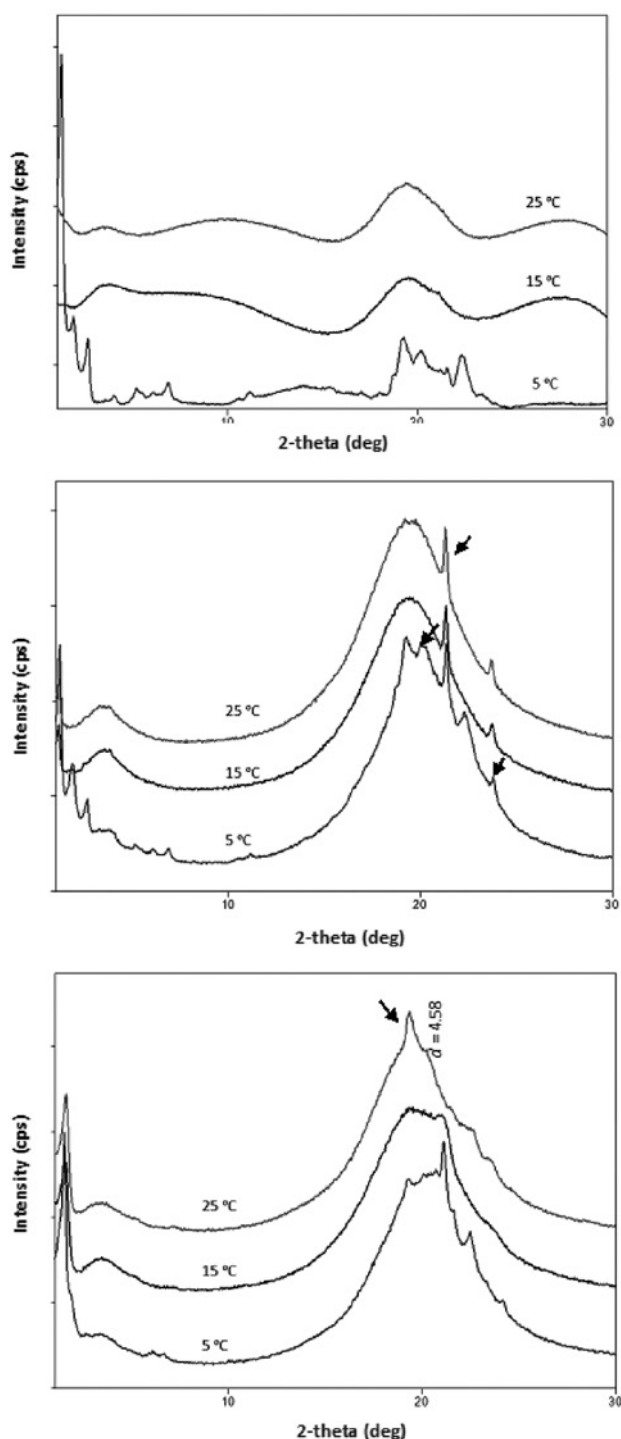


FIGURE 3. Polymorphism diagram of (A) PBA, (B) SFW and (C) MGHO superolein oleogels at preparation temperatures of 5, 15 and 25 °C on day 1.

as only an amorphous peak at approximately 4.5 Å was observed in the WAXS region for PBA oleogels. Pradhan *et al.* (2014) also reported the detection of this single peak at approximately 20°(2 θ)

in palm oil-based organogels, which suggested the amorphous nature of the organogel. Bot *et al.* (2009) reported that two peaks associated with liquid phases for a self-assembly organogel, which was similar to those of the PBA oleogels stored at 15 and 25 °C. This finding indicated that the organogelation event that took place in the formation of PBA oleogels also via self-assembly.

At 5 °C, a few crystalline peaks were observed at WAXS and SAXS, indicating the presence of crystalline particles in the oleogel system due to the crystallization of the superolein. Figure 3 shows that 3 wide-angle peaks were present: the peak that appeared at $d = 4.61$ Å indicated the detection of β polymorphic form crystals; whereas the peaks detected at $d = 4.31$ Å and 3.89 Å were due to the presence of the β' polymorphic form of crystals (D'Souza *et al.*, 1990; deMan, 1992; Peyronel and Marangoni, 2013).

Figure 3B shows how the XRD patterns for SFW oleogels displayed a combination of amorphous and crystalline behavior. Three persistent peaks were detected at 4.60 Å, 4.15 Å and 3.74 Å for SFW peaks stored at all storage temperatures. Peaks with exactly the same d -spacing were also reported by Ögütçü *et al.* (2015) in which wax oleogels from beeswax and rice bran wax also produced peaks at similar WAXS regions, indicating the presence of the β' polymorphic form of crystals. Tavernier *et al.* (2017) also reported similar peaks which represented β' crystals associated with high-melting wax. This explains the detection of these peaks in almost all the literature concerning wax oleogels. In contrast, the peak detected at 4.60 Å could also indicate the presence of β polymorphs of crystal (AOCS, 2017c). However, this peak was just a broad peak at storage temperature of 15 and 25 °C, which indicated an amorphous scattering contributed by the superolein (Bot *et al.*, 2009; Da Pieve *et al.*, 2011). In contrast, the peak at d -spacing of 4.60 Å for 5 °C storage was sharp, which indicated the presence of β polymorphs of crystals at the low temperature. These observations indicated that SFW oleogels consist mainly of β' polymorphs. Additionally, peaks at 4.42 Å and 3.89 Å were detected in PBA and SFW oleogels stored at 5 °C, indicating β' polymorphic crystals due to the crystallization of the superolein. This shows that palm superolein crystallization did not interfere with the gel structure presence in both PBA and SFW

oleogels. On the other hand, this observation also suggests that superolein is fully melted at temperatures above 15 °C.

MGHO oleogels showed a complicated pattern with a combination of amorphous and crystalline behaviour. Unlike PBA and SFW oleogels, MGHO oleogels did not show a consistent trend when stored at different temperatures, indicating that the crystal packing in MGHO oleogels was different when stored at different temperatures. At 25 °C, a major peak at 4.58 Å, and some minor peaks at 4.38 Å, 3.93 Å, and 3.78 Å were observed. Da Pieve *et al.* (2011) reported a very similar X-ray diffraction pattern in cod liver oil monoglyceride oleogel. These peaks are due to the in-plane ordering of monoglyceride aliphatic chains into the β -phase. This makes sense as the peak at 4.58 Å was sharp, different from those amorphous peaks detected in SFW and PBA oleogels at 15 and 25 °C.

At 15 °C, only some small peaks at 4.57 Å, 4.21 Å and 3.92 Å were observed. The intensity of the 4.57 Å peak was lower than that at 25 °C, which revealed a decrease in the β polymorphic form crystals. The detection of very-low-intensity peaks at 4.21 Å and 3.92 Å indicated the presence of a low amount of β' crystals. This finding indicates that the in-plane ordering of the MGHO aliphatic chains is predominantly in triclinic configuration with some crystals packed in an orthorhombic configuration. At 5 °C, MGHO oleogels showed a similar XRD pattern to SFW and PBA oleogels, indicating that the crystal polymorphism in all these oleogels was mainly contributed by the superolein crystals at 5 °C.

The molecules of the MGHO interacted very closely with superolein molecules due to their high solubility in the superolein, thus displaying different interactions at different temperatures. Their interactions could be associated with the crystallization properties of TAGs in the superolein. At 25 °C, the superolein remained liquid, thus resulting in minimal interactions. During this period, the MGHO molecules self-assemble into inverse lamellae, forming α -crystals (hexagonal packing) before transition to sub- α crystals (orthorhombic chain packing) (Chen and Terentjev, 2009). At low temperatures, the higher melting TAGs in superolein interacted with the MGHO molecules, packing themselves into crystals with different polymorphism compared to the oleogels stored at 25 °C. This observation was different from the findings reported by Da Pieve *et*

al. (2011), whereby no diffraction difference was detected when XRD patterns were recorded at 4 and 20 °C in a cod liver oil oleogel system. This could be due to the properties of the oil used, as superolein oil consists of some saturated components, similar to those in MGHO, thus affecting the packing of molecules in crystal formation. The measured diffraction values for the PBA, SFW and MGHO oleogels are summarized in Table 2.

According to Peyronel and Marangoni (2013), the Bragg peak position in the SAXS region characterizes the longitudinal packing of fat molecules, indicating the size of bilayers or lamellae formed by stacking (001) planes. In the SAXS region, single, broad, weak peaks were observed for oleogels stored at 15 and 25 °C, indicating no obvious longitudinal packing and only liquid/amorphous phases in the PBA oleogel system. At 5 °C, a peak of 47.71 Å corresponded to 2L of the (001) plane, showing lamellae of approxi-

TABLE 2. Summary of the diffraction values measured in the PBA, SFW and MGHO oleogels at 5, 15 and 25 °C on day 1

Gelator	Temperature (°C)	SAXD (Å)	WAXD (Å)
PBA	5	47.71 (001) _{IV64}	4.61, 4.31, 3.89
		33.90	
		17.11 (003) _{IV64}	
		12.89	
	15	26.0	4.48
	25	24.5	4.53
SFW	5	69.2 (001) _{SFW}	4.61, 4.42, 4.15, 3.89, 3.73
		47.6 (001) _{IV64}	
		33.9 (002) _{SFW}	
		23.5 (003) _{SFW}	
		12.9	
	15	69.8 (001) _{SFW}	4.58, 4.16, 3.74
	25	23.2 (003) _{SFW}	
		71.2 (001) _{SFW}	4.61, 4.16, 3.74
		23.9 (003) _{SFW}	
MGHO	5	59.0	4.60, 4.29, 4.40, 3.94, 3.68
	15	57.0	4.57, 4.21, 3.92
	25	55.3	4.58, 4.38, 3.93, 3.78

NOTE: SAXD = small-angle X-ray diffraction, WAXD = wide-angle X-ray diffraction, PBA = polyglycerol behenic acid ester, SFW = sunflower wax, MGHO = monoacylglycerol with high oleic, IV64 = palm olein of IV64

mately 47.71 Å (Da Pieve *et al.*, 2011). Weaker peaks at 33.90 Å and 17.11 Å corresponded to higher-order reflections of the same repeating distance.

At 5 °C, the first peak observed in SFW oleogels was at 47.60 Å, followed by a higher-order reflection at 33.86 Å, which corresponded to a 2L lamellae of the (001) planes. A consistent broad peak at 23.5 Å across all temperatures reflected the formation of a lamellae layer by the SFW. In MGHO, a single sharp peak in the SAXS region which increased with the decrease in storage temperature, with *d*-spacing values increasing from 55.31 Å to 57.02 Å and 59.04, when the temperature was reduced from 25 , to 15 and 5 °C, respectively. These findings indicated the formation of a thicker lamellae layer with a reduction in storage temperature.

3.4. Effects of storage temperature on microstructure

Figure 4 illustrates the morphology of the superolein oleogels on day 1 of storage. Only day 1 images were discussed due to the lack of noticeable differ-

ences over time. Obviously, the gel network present in PBA oleogels was a non-crystalline structure, formed via the self-assembly of the PBA molecules, creating a self-supporting gel network to entrap the superolein. Under bright-field microscopy, the oleogels displayed a tiny worm-like entangled network at 15 and 25 °C, aligning with XRD results, which showed only an amorphous peak for PBA oleogels. At 5 °C, the worm-like structure was not noticeable, but many spherulitic crystals were visible. These crystals were arranged in a regular manner, indicating that the crystals were separated from one another by the “invisible” non-crystalline gel structure, which was less visible under bright-field microscopy. This confirms the crystallization of the higher melting fraction in the superolein within the PBA gel at 5 °C.

From the microscope images of SFW oleogels illustrated in Figure 4D-F, fewer crystals were visible at lower temperatures, which was contrary to expectations from SFC results. This counter-intuitive observation is due to the limitations of bright-field microscopy, whereby the actual presence of

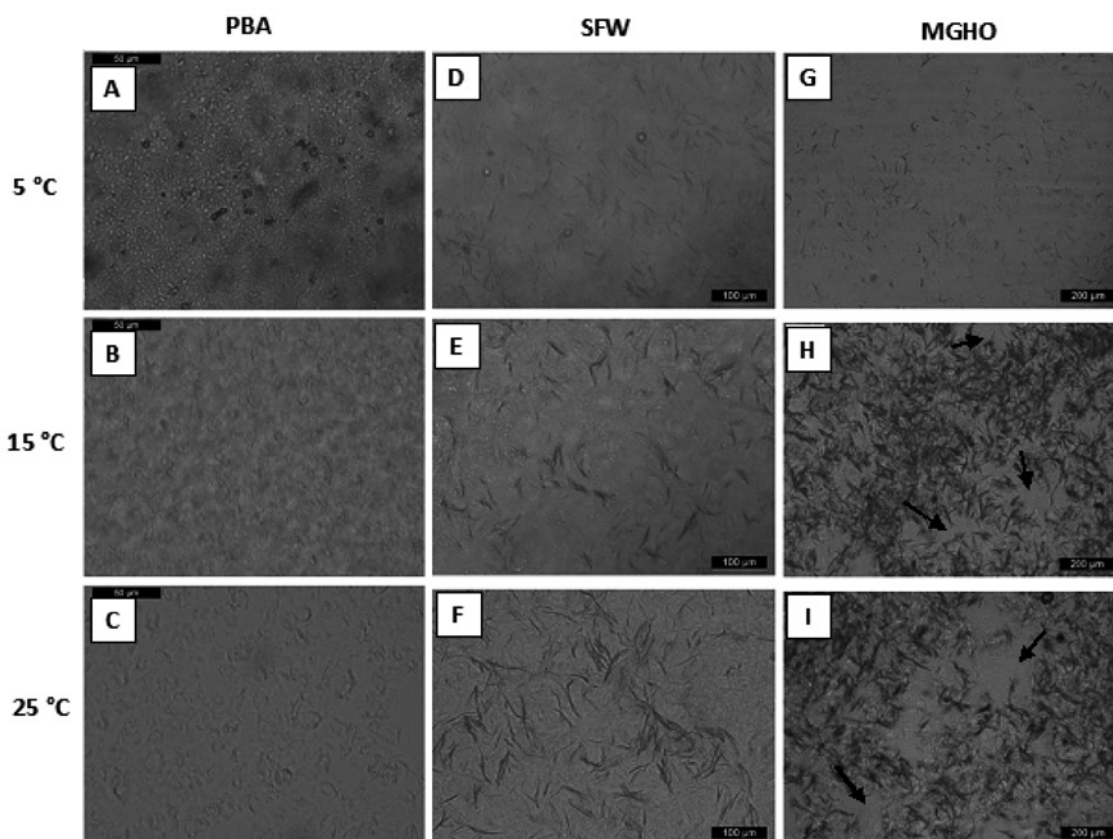


FIGURE 4. Morphology of superolein oleogel from PBA (10x magnification), SFW (20x magnification) and MGHO (50x magnification) stored at 5 °C, (B) 15 °C, and (C) 25 °C on day 1 of preparation

crystals cannot be clearly seen due to the presence of thick background crystals. The gel structure was more visible at higher temperatures when the oil was liquid, allowing better light transmission (Microscopy, 2013).

The crystals shown in the microphotographs of MGHO oleogels stored at 5 °C were less visible compared to those stored at 15 and 25 °C (Figure 4G-I). The actual quantity of crystals could not be reflected on the bright-field microscopy for the same reason as discussed previously. In comparison, the gel network in the oleogel at 5 °C was more compact and arranged in a more uniform order compared to the oleogels stored at higher temperatures. Some voids between the crystals were observed in the MGHO oleogels stored at 15 and 25 °C. Overall, the superolein MGHO oleogels exhibited needle-like structures at all storage temperatures. This finding is similar to other studies that involved the use of monoglyceride (MG) gelators (Bin Sintang *et al.*, 2017; Da Pieve *et al.*, 2010; Kesselman and Shimoni, 2007).

3.5. Effects of storage temperature on stability

The oil-released percentages reflecting oleogel stability are summarized in Figure 5. At 5 °C, the oil released significantly increased with a longer preparation day for PBA superolein oleogels from 5.0 to 7.5%. For SFW oleogel, only 4.5% oil release was observed after completing the storage duration. For MGHO oleogel, the oil release for MGHO oleogels was significantly higher than those with PBA and SFW oleogels, ranging from 8.1 to 14.3%.

At 15 °C, SFW oleogels were completely stable during storage. A low amount of oil release of 3.6 to 6.8% was observed for PBA oleogel. At 25 °C, PBA oleogels released more oil compared to the amounts released at lower temperatures. For MGHO oleogel, the oil released significantly increased with higher temperatures. The oil release was the highest at 25 °C, whereby almost 50% of the oil was released out from the structure.

In general, the centrifugal force applied in this analysis was able to separate the free liquid oil that was bound to the crystal structure (Kanagaratnam *et al.*, 2013). These results indicated that SFW gelator had the highest capacity to hold liquids within its structure. The gelator was very different in composition compared to the liquid oil, consisting of mainly esters of

fatty acids with fatty alcohols (Carelli *et al.*, 2002). The gelator was able to form a homogenous and continuous structure capable of holding the oil within it.

MGHO gelator was not effective in forming a strong structure to hold the liquid oil even when a high dosage of 8%w/w gelator was used. This is probably due to the composition and structure of the

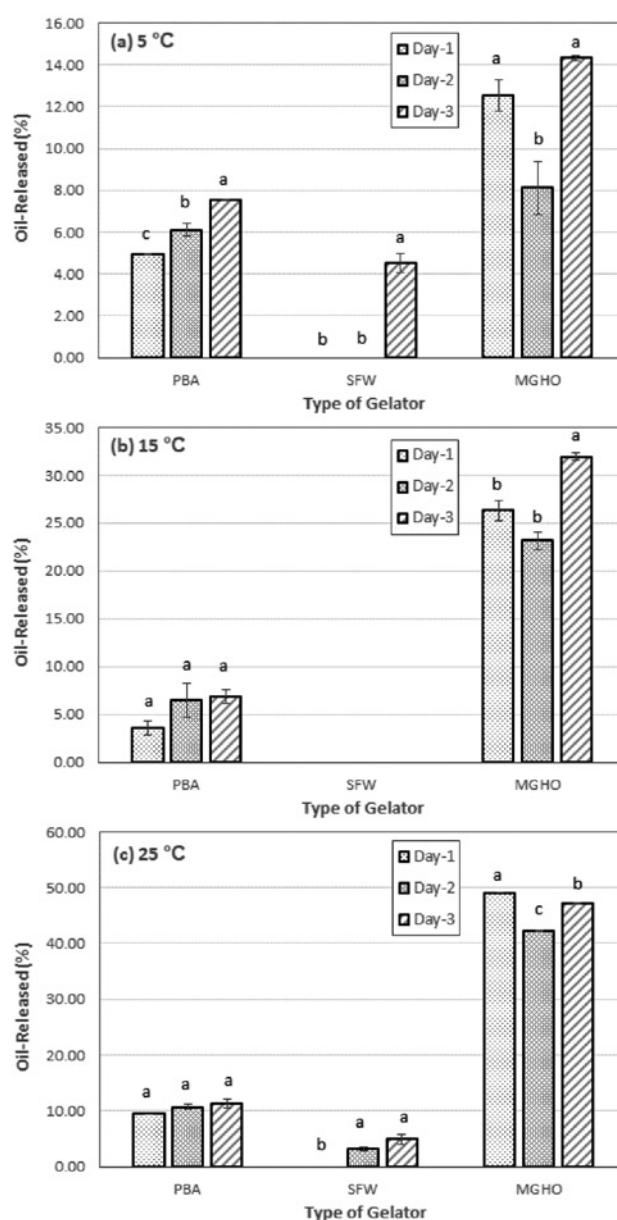


FIGURE 5. Comparison of percentage of oil released from oleogels from PBA, SFW and MGHO gelator at (A) 5 °C, (B) 15 °C and (C) 25 °C.

Each value represents the mean $\pm$ standard deviation of triplicate analyses. Significance testing was conducted between the same kinds of oleogels prepared on different days of preparation.

Different letters indicate significant differences at $p < 0.05$ according to the Tukey's test.

MGHO gelator, with fatty acid chain lengths of C16 and C18, which are very similar to the superolein. At low temperatures, the MGHO molecules and the higher saturation component in superolein underwent nucleation and aggregated into larger crystals. Voids were formed between the large crystals without a continuous structure (Figure 4), in which MGHO appeared as large dendritic crystals in the superolein without forming a tight network to hold the liquid oil in its structure. These voids filled with the less saturated liquid oil, easily released from the structure when centrifugal force was applied. A similar explanation was reported by Patel *et al.* (2015), as bee wax and fruit wax formed loose entanglements of large crystals that could sustain lower magnitudes of stress. Thus, these oleogels had lower values of dynamic moduli in the linear response region compared to the other oleogels from natural waxes, such as SFW and carnauba wax. Additionally, MGHO oleogels present as β -crystalline phase exhibit large crystal aggregation which may also affect their oil binding capacity (Bin Sintang *et al.*, 2017), thus making them least stable compared to the other oleogels.

Interestingly, MGHO gelators showed very high hardness but still exhibited very poor stability. A study conducted by Razul *et al.* (2014) indicated that harder surfaces retain less oil than softer surfaces, meaning that harder surfaces tend to have lower oil-binding capacities, which was in-line with our findings. At 25 °C, the gelator molecules were highly soluble in the liquid oil due to their similarity in fatty acid composition. The gelator was not able to form a strong structure, which resulted in a very high amount of oil released.

4. CONCLUSIONS

In conclusion, both the storage temperature and duration affected the physical and microstructure properties of the superolein oleogels. The effect of temperature was very significant, mainly due to the tendency of the superolein to crystallize within the oleogel structure at low temperatures. Occasionally, the gelator may also play an important role in affecting the oleogel properties, as the gelator molecules interacted differently with the oil molecules when stored at different temperatures. The analyses on the microstructure, such as crystal polymorphism and morphology studies, indicated that the MGHO gela-

tor formed different kinds of crystals when stored at different temperatures, and rearrangement of crystals also occurred during the 3-day storage. The hardness, SFC and morphology findings indicated that the storage duration also affected the properties of superolein oleogels due to the slow crystallization of superolein, detected mainly at 15 °C, when SFW and MGHO were used. Theoretically, the physical, thermal, rheological and microstructure behaviors can affect the stability of oleogels. Oleogels with higher hardness and stronger rheological properties tend to form more stable gels. However, we found that oleogels with high hardness, SFC and melting properties may not necessarily show good stability, *e.g.*, MGHO oleogel showed the highest hardness but it was the least stable. The stability of oleogels is highly dependent on their microstructure behavior, preferring to form a continuous, uniform, build of a strong gel network which is able to entrap liquid oil efficiently within its structure. Therefore, SFW was found to be the best gelator, as it formed uniform and continuous crystalline structures. In contrast, MGHO formed a loose entanglement of crystals, with the appearance of some voids between the crystals that easily oiled out during centrifugation. Other than the storage temperature and duration, the oleogel composition made up of the gelator and liquid oil may also affect the microstructure behavior and the stability of oleogels. Therefore, future research can explore the choice of liquid oil and the concentration of gelator in oleogel formation, as understanding the relationship between oleogel composition and oleogel stability is crucial for practical applications.

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AUTHORSHIP CONTRIBUTION STATEMENT

MH Saw: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft. WH Lim: Conceptualization,

Methodology, Writing – review & editing. CB Yeoh: Conceptualization, Investigation, Writing – review & editing. E Hishamuddin: Conceptualization, Investigation, Writing – review & editing. S Kanagaratnam: Funding acquisition, Methodology, Writing – review & editing. NA Mohd Hassim: Investigation, Writing – review & editing. NH Ismail: Investigation, Writing – review & editing. CP Tan: Conceptualization, Methodology, Writing – review & editing.

CONFLICT OF INTEREST

All the authors declare that there is no conflict of interest in this work.

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