

PHYSICOCHEMICAL AND RHEOLOGICAL PROPERTIES OF RED PALM OLEIN OLEOGELS MADE WITH BEESWAX AS THE OLEOGELATOR

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ABSTRACT

Red palm olein (RPO) is a source of carotenoids that can act as provitamin A and pigment. RPO is liquid at room temperature and heating degrades carotenoids easily. Modifying RPO into a solid by oleogelation is essential to expand its application in food products. This study aimed to identify the physicochemical and rheological properties of RPO oleogels, using beeswax as the oleogelator, including crystal morphology and formation, solid fat content, total carotenoid content, and colour analysis. This study consisted of three stages: characterisation of the raw materials, preparation of RPO oleogels (0%-10% beeswax), and characterisation of the RPO oleogels. RPO starts to form as a solid at 3% beeswax with a melting point of $40.61 \pm 1.73^{\circ}\text{C}$ (solid at room temperature) and 557.73 ± 10.28 ppm of total carotenoid. Furthermore, increasing the beeswax concentration increased the compacity of the needle-shaped crystal structure, which has an impact on the rheological properties of the oleogels, including the higher linear viscoelastic region, yield stress, melting point, and stability against frequency and temperature. RPO oleogel viscoelasticity showed a positive relationship with solid fat content. However, increasing beeswax concentration significantly decreases the total carotenoids of RPO oleogels, while increasing the lightness and yellowish colour of the oleogels.

Keywords: beeswax, oleogel, red palm olein.

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INTRODUCTION

Red palm olein (RPO) contains higher carotenoids, especially 85% of β -carotene, than other palm oil products (Eze *et al.*, 2021; Nainggolan and Sinaga, 2021). RPO is produced from crude palm oil containing 500-700 ppm of carotenoids through degumming, deacidification, and fractionation. Technically, its processing is conducted without bleaching or deodorisation, enabling the retention of carotenoids in crude palm oil (Dewi *et al.*, 2023;

Purnama *et al.*, 2020). Carotenoids in RPO increase retinol levels in the blood serum, which justifies their use in cases of vitamin A deficiency (Harlen *et al.*, 2018; Loganathan *et al.*, 2017). Treatment of vitamin A deficiency is important because it causes metabolic disorders, metaplasia (changes in cell function), infectious diseases, immunoglobulin dysregulation, anaemia, promyelocytic leukaemia, xerophthalmia (failure to produce tears), cancer development, and death (Carazo *et al.*, 2021; Surman *et al.*, 2020).

RPO consists of 42.23% palmitic acid (C:16-1), 41.58% oleic acid (C:18-1), and 10.71% linoleic acid (C:18-2), and for this reason, it is liquid at room temperature (Marliyati *et al.*, 2021; Nainggolan and Sinaga, 2021). The carotenoids in RPO begin to degrade significantly at 60°C , leading to its limited application in food products (Sarah, 2018). Therefore, it is necessary to modify RPO to make

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it solid at room temperature while maintaining its carotenoid content. A modified RPO, which is in solid form, can be formulated for several food products that require raw fat materials, such as margarine, spread, and shortening (Hasibuan *et al.*, 2019; Wang *et al.*, 2021).

Oleogelation is an alternative to the conventional method that facilitates the mixing of oil and oleogelator through heating, followed by crystallisation through cooling to produce an oleogel. This process can minimise changes in the fatty acid composition and the formation of *trans* fatty acids by selecting an effective oleogelator (Samateh *et al.*, 2018). Natural waxes can form fat crystal networks at concentrations of less than 10% (Morales *et al.*, 2023; Sena *et al.*, 2022). Among natural wax, beeswax is often used in oleogelation, considering that Indonesia is among the world's top 20 largest beeswax-exporting countries. In addition, the beeswax market has experienced a significant growth in the last five years and is predicted to continue to grow until 2029 (SEAIR, 2023).

Oleogels are viscoelastic mixtures of liquid and solid lipids that possess specific properties that change owing to certain forces or disturbances (Han *et al.*, 2022; Naeli *et al.*, 2021; Pang *et al.*, 2020; Xu *et al.*, 2022). This has provoked research interests in the rheological analysis of viscoelastic materials. However, the profiles and rheological characteristics of RPO oleogels remain unknown. Therefore, this study aimed to identify the rheological properties of RPO oleogels using a beeswax oleogelator. The rheological properties were confirmed by morphological analysis and solid fat content (SFC). Morphological analyses were performed to observe the crystal morphology and formation of the oleogel. SFC is one of the parameters commonly used by the food industry in developing fat-based foods. In addition, considering the high carotenoid contents in RPO, this study determined the carotenoid content and colour of RPO oleogels at different beeswax concentrations.

MATERIALS AND METHODS

Samples

RPO was obtained from Carotino Sdn. Bhd. (Johor, Malaysia), and the beeswax was obtained from Babybees (Jakarta, Indonesia). The RPO is liquid at room temperature. It contained 60.93% unsaturated fatty acids mainly oleic and linoleic acids and 0.04% free fatty acids. The beeswax is solid at room temperature. It had 5.55 mg KOH/g of acid value, 97.55 mg KOH/g of ester value, 61°C slip melting point, and 110.04 mg KOH/g of saponification value.

Reagents

Wijs reagent, potassium iodate ($\geq 99.5\%$), potassium iodide ($\geq 99.5\%$), sodium thiosulfate (99.5%-101.0%), glacial acetic acid ($\geq 99.8\%$), ethanol ($\geq 99.5\%$), sodium hydroxide ($\geq 97.0\%$), cyclohexane ($\geq 99.5\%$), phenolphthalein ($\geq 99.9\%$), amylum indicator ($\geq 99.9\%$) and ethanol (95.1%-96.9%) were purchased from Merck (Darmstadt, Germany). Isooctane ($\geq 99.0\%$) was purchased from SmartLab Indonesia (Jakarta, Indonesia), while distilled water was procured from a laboratory in the Department of Food Science and Technology at IPB University (Bogor, Indonesia). Demineralised water was procured by PT Laboratorindo Jaya Perkasa (Sidoarjo, Indonesia).

RPO Characterisation

RPO characterisation followed the standard method of the Indonesian National Standard 9098:2022 for Red Cooking Oil (BSN, 2022), concerning colour by Model F Lovibond Tintometer (England), odour and taste with organoleptic test, moisture content and volatile matter by UN55 Memmert Oven (Germany), iodine number, peroxide value, free fatty acid content, and total carotenoids by Uvmini-1240 Shimadzu UV-Vis spectrophotometer (Germany), and pelican oil. Pelican oil contains minerals that cannot be saponified in an alkaline alcohol-water solution.

Oleogelation

RPO and beeswax with a total mass of 10 g were heated at 65°C for 20 min in a LabTech LSB-015S shaking water bath (Indonesia). Subsequently, the mixture was cooled to 5°C for 24 hrs (Kamali *et al.*, 2019). The beeswax concentrations were 0%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, and 10% in duplicates.

Critical Gelling Concentration

Determination of critical gelling concentration was carried out visually by inverting the oleogel test tube at 25°C. The formation of a solid phase in the oleogel was indicated by a non-flowing oleogel (Flöter *et al.*, 2021).

Morphology Analysis

Melted oleogels were dropped onto a preheated slide and immediately covered with a cover slip. It was stored at 5°C for 24 hrs. The crystal morphology of the RPO oleogels was observed using a CX31-P Olympus polarised microscope (Japan), at 1000× magnifications (Pang *et al.*, 2020).

Rheology

The rheological properties were analysed using Anton Paar MCR 92 rheometers (Austria). Rheological analysis consisted of amplitude, frequency, and temperature sweep. An amplitude sweep was carried out at a strain of 0.01%-100.00%, with angular frequency of 10 rad/s at 25°C. A frequency sweep was performed in the linear viscoelastic region (LVR) with a frequency of 0.1-100.0 rad/s and a temperature of 25°C. The temperature sweep was carried out 25°C-90°C with 5°C/min and an angular frequency of 10 rad/s at linear viscoelastic region (LVR) (Barroso *et al.*, 2022). This analysis was performed using PP50/S (beeswax and oleogel 1%-10% beeswax) and CP50-1 (RPO and oleogel 0% beeswax) geometries.

Melting Point

The melting point was determined from the result of the temperature sweep in rheology analysis. This melting point is an estimated melting point that was determined by the crossover point of G' and G'' , affected by temperature (Atik *et al.*, 2022).

Solid Fat Content

Solid fat content (SFC) was analysed by MQ20 Minispec nuclear magnetic resonance (NMR) analyser (Germany). The sample was heated at 100°C for 15 min in a water bath. The water bath was then turned off, and the sample was left in the water bath for 30 min. They were re-heated in a water bath at 60°C for 5 min and transferred to a water bath at 0°C for 60 min. Samples were analysed at temperatures of 0°C, 10°C, 20°C, 30°C, 40°C, 50°C, 60°C, 70°C, and 80°C or until the sample showed SFC of 0% (AOCS Official Method Cd 16b-93, 2022).

Total Carotenoid

The total carotenoid content was analysed using a UVmini-1240 Shimadzu UV-Vis spectrophotometer (Germany). Samples (0.1-0.5 g) were diluted with isooctane in a 25 mL volumetric flask, then analysed in a UV-Vis spectrophotometer at a wavelength of 446 nm (ISO 17932, 2011).

Colour Characterisation

The colour was analysed using a Minolta CR-400 chromameter. The chromameter was calibrated on standard white and black plates. Colour analysis was performed by placing a homogeneous sample in a cylindrical tube with a diameter of 2 cm and a height of 1 cm (Papadaki *et al.*, 2020).

Statistical Analysis

The experimental design of this study was a completely randomised design (CRD) with two treatment replicates, and all tests were repeated in duplicate. Data were analysed by analysis of variance (ANOVA) using the IBM SPSS Statistics 25 software with an alpha of 5%.

RESULTS AND DISCUSSION

Characteristics of Red Palm Olein

Table 1 shows that the RPO samples fully complied with the quality requirements of Indonesian National Standard (SNI 9098:2022) for Red Cooking Oil. The samples were of high quality and were made from high-quality raw materials; thus, they could be used as food ingredients.

TABLE 1. RPO CHARACTERISTICS BASED ON INDONESIA NATIONAL STANDARD (SNI 9098:2022)

Parameter	Quality requirements on SNI 9098:2022	Analysis results
Colour (Lovibond unit)		
Red	≥ 10	15.07 ± 0.06
Yellow	≥ 10	10.03 ± 0.06
Odor	Normal	Normal
Taste	Normal	Normal
Moisture content and volatile matter (%)	≤ 0.15	0.06 ± 0.02
Iodine number (g iod/100 g)	≥ 56.00	60.93 ± 0.52
Peroxide value (meq O ₂ /kg)	≤ 10.00	7.85 ± 0.32
Free fatty acid content (%)	≤ 0.50	0.04 ± 0.00
Total carotenoids (mg/kg)	≥ 400.00	657.24 ± 15.67
Pelican oil	Negative	Negative

Critical Gelling Concentration

As shown in *Figure 1*, the RPO oleogels with 0%, 1%, and 2% beeswax flowed to the bottom of the tube due to gravity, indicating that the oleogels were liquid and could flow without additional force. However, the control sample (without beeswax) moved completely to the bottom of the tube, and no oleogel remained at the top. Intriguingly, the RPO oleogel with 1% beeswax left residual oleogel on the inner tube wall. Visually, the remaining sample left in the tube appeared orange at the top of the test tube of the sample containing 2% beeswax, indicating it contained more residue than that containing 1% beeswax. A slower flow time indicated higher viscosity and higher resistance because it has more friction (Jia *et al.*, 2022).

In contrast, RPO oleogel with 3% to 10% beeswax did not flow to the tube bottom after inversion. This indicated that the sample was solid at room temperature. Higher beeswax concentrations resulted in more solid oleogels (Han *et al.*, 2022; Pang *et al.*, 2020; Zbikowska *et al.*, 2022). About 3% beeswax is the minimum amount required to make the RPO oleogel solid at room temperature. Therefore, the critical gelling concentration of the RPO oleogel with beeswax as oleogelator is 3%.

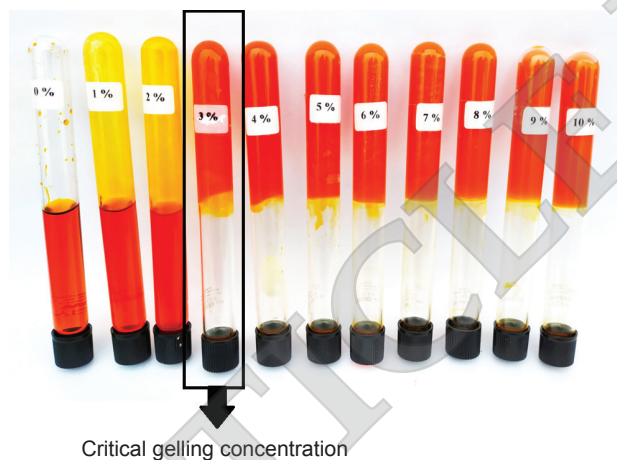


Figure 1. Critical gelling concentration of RPO oleogel.

Morphology Analysis

Figure 2 shows that the RPO oleogels had needle-shaped crystals. Its crystal morphology is characteristic of an oleogel that uses beeswax as the oleogelator (Chen *et al.*, 2021; Gómez-Estaca *et al.*, 2019; Pang *et al.*, 2020). RPO oleogel with 3%-10% beeswax has a compact crystal structure. Higher beeswax concentration resulted in denser crystal structures. Our results are consistent with that of Pang *et al.* (2020) in that the crystals are denser with higher concentration of beeswax in oleogels, either with camellia oil, sunflower oil, flaxseed

oil or soybean oil. RPO oleogel with 10% beeswax is known to contain fluorescent molecules in the beeswax.

In contrast, RPO and RPO oleogel with 0% beeswax (control) had no crystal structure, therefore they were liquid at room temperature. Notably, the control oleogel (with 0% beeswax) was an RPO which underwent an oleogelation process without adding the beeswax. The RPO oleogels with 1% and 2% beeswax had needle-like crystals but did not have a compact structure. This result is consistent with the observations of the critical gelation concentrations, which indicated that the transition concentration was 3%. When viewed through a polarised microscope, compact crystal structures were formed at the transition concentrations. The crystal structure of beeswax had trapped the RPO into a solid form.

Rheology

Amplitude sweep results. The linear viscoelastic region (LVR) and yield stress were determined using an amplitude sweep. The maximum shear strain value at the LVR (LVR limit) was determined based on the shear strain value at the storage modulus (G'), loss modulus (G''), or complex modulus (G^*) with a 5% difference from the shear strain value using a horizontal line plot (ISO 6721-10, 2015). The LVR is the range in which at a given disturbance (shear strain or shear stress) on the sample, it does not damage the structure of the samples (Mezger, 2021). In this study, it was evident that beeswax had significantly affected the LVR (*Figure 3*). Higher levels of beeswax resulted in a wider range of LVR. A wider LVR indicates that the sample is more resistant to disturbances in the form of shear strain. Beeswax increases the strength and stability of the three-dimensional bonds to stabilise the RPO structure against shear strain treatment (Chai *et al.*, 2022; Espert *et al.*, 2022). However, the LVR for the RPO oleogels with 0% and 1% beeswax could not be identified because they were Newtonian fluids. The viscosity of a Newtonian fluid is not affected by the force applied to the flow, and a very small shear strain can cause sample deformation (Alexander, 2017; Gagliardi, 2020; Hariyadi *et al.*, 2019).

The yield stress is the minimum force required to flow a material, such as a liquid generally possessed by non-Newtonian fluids (Hariyadi *et al.*, 2019). This analysis revealed that increased beeswax oleogelator levels increased the yield stress (*Figure 4*). The yield stress is associated with the length of the saturated fatty acid chains in the beeswax and the crystal structure of the oleogel. Beeswax mainly contains C24 to C36 fatty acids, most of which are saturated fatty acids (approximately 85%). However, the dominant saturated fatty acids chain length was C14-C31, with a long-chain of

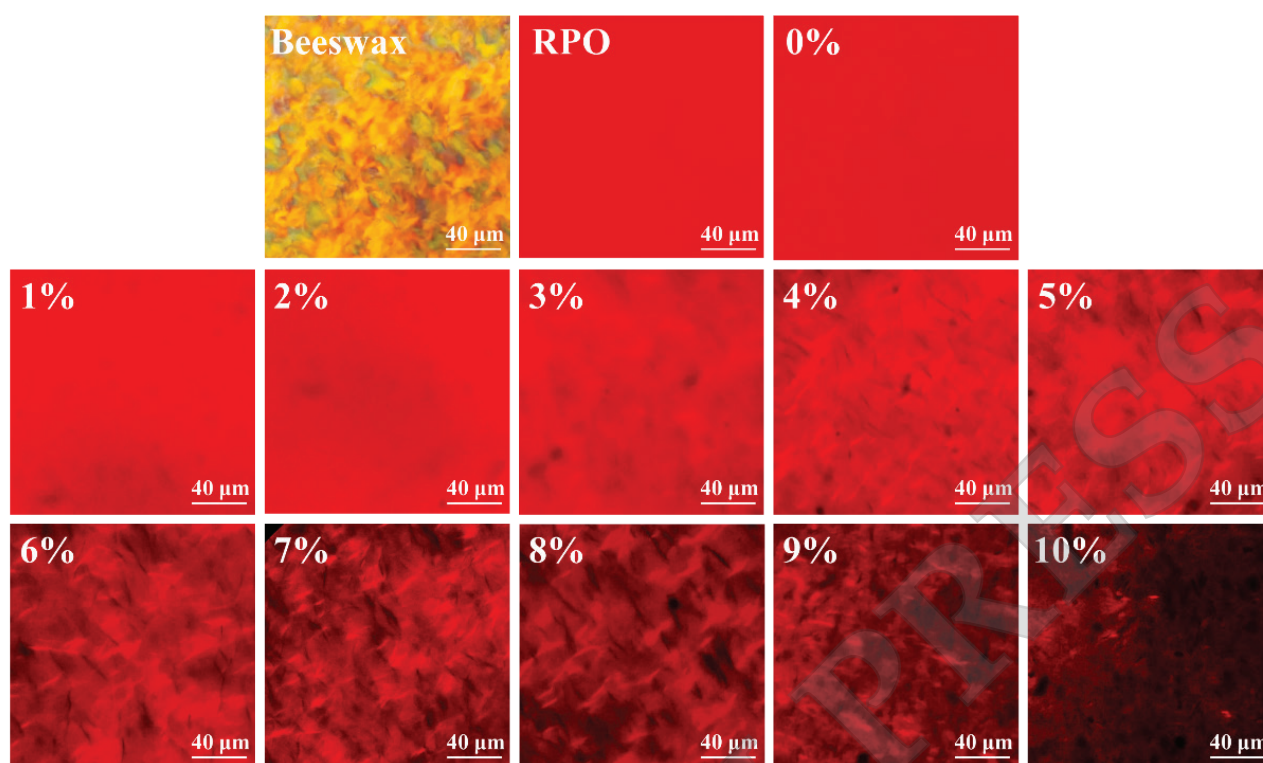


Figure 2. Needle-shaped crystal of beeswax, RPO, and RPO oleogels.

even-numbered palmitate wax esters and fatty acids (Chasan *et al.*, 2021; Doan *et al.*, 2017; Szulc *et al.*, 2020). As explained in the morphology analysis section, the higher beeswax concentration caused a denser crystal structure. A denser crystal structure resulted in a higher elastic modulus value; therefore, a stronger force was required to destroy the oleogel structure. Therefore, the yield stress increased as the beeswax concentration increased.

Frequency sweep results. Changes in the frequency given to the sample markedly alter storage modulus (G') and loss modulus (G''). Values are specific to each sample and frequency. In addition, the G' and G'' have the same value at the crossover point. The crossover point is indicated by the angular frequency required to achieve a loss factor of 1 (Sivakanthan *et al.*, 2023). The loss factor is the ratio of loss modulus to storage modulus. In the case of RPO oleogels with 0%-3% beeswax, the higher concentration of beeswax resulted in a higher crossover point (Figure 5). A higher crossover point indicates that a higher frequency is required to change the RPO oleogel phase, from the solid to the liquid phase. In addition, the higher the concentration of beeswax, the more stable the RPO oleogel structure (Doan *et al.*, 2017; Kwon and Chang, 2022; Sivakanthan *et al.*, 2023).

Moreover, G' is higher than G'' at frequency below the crossover point, suggesting that the sample is solid. Conversely, above the crossover point, G' was lower than G'' , indicating that the

sample was liquid. Previous studies have discovered different types of oleogels, including walnut oleogels with monoglycerides, sunflower seed oleogels with carnauba wax, hazelnut oleogels with carnauba wax and olive oleogels with carnauba wax, with similar results (Pehlivanoglu *et al.*, 2021; Sun *et al.*, 2022).

In addition, the higher concentration of beeswax had increased G' and G'' . However, the crossover points of the RPO oleogel with 4%-10% beeswax could not be identified. Oleogels with 4%-10% beeswax had a crossover point of more than 100 rad/s, exceeding the detection limit of the rheometer used. Thus, they were not analysed further in this study. However, these results demonstrated that the angular frequency range of 0.10-100.00 rad/s was unable to change the 4%-10% oleogel phase from solid to liquid.

Temperature Sweep Results. The addition of the beeswax oleogelator to the formulation of the RPO oleogel was aimed at increasing the melting point of RPO. Beeswax, which has elevated long-chain fatty acids, immobilises RPO fatty acid molecules in a three-dimensional matrix, formed from the beeswax needle crystals. Beeswax can transform RPO from liquid to solid at room temperature. In this study, the melting point of RPO had increased significantly with the addition of beeswax (Table 2).

The RPO oleogels with 0% and 1% beeswax had melting points of less than the room temperatures (<25°C). It indicated that the oleogels were liquid at room temperature. The RPO oleogel with

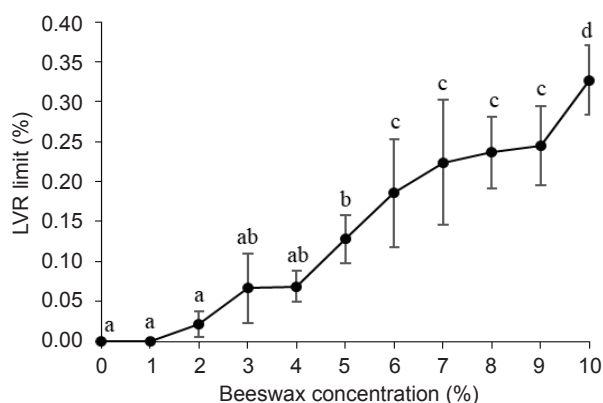


Figure 3. Linear viscoelastic region (LVR) limit of red palm olein oleogels. Distinct letters show significant differences between the LVR limit of the samples (two-way ANOVA; Duncan test: p -value ≤ 0.05).

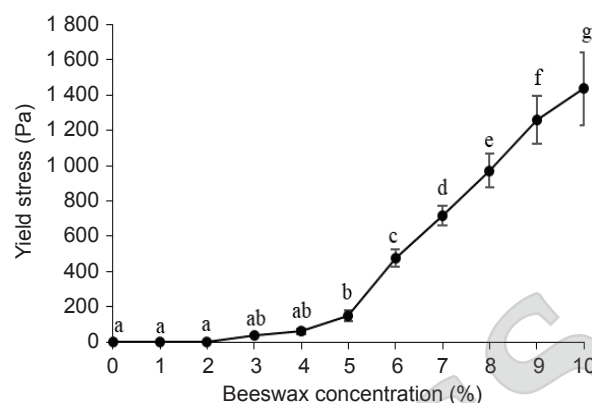


Figure 4. Yield stress of red palm olein oleogels. Distinct letters show significant differences between the yield stress of the samples (two-way ANOVA; Duncan test: p -value ≤ 0.05).

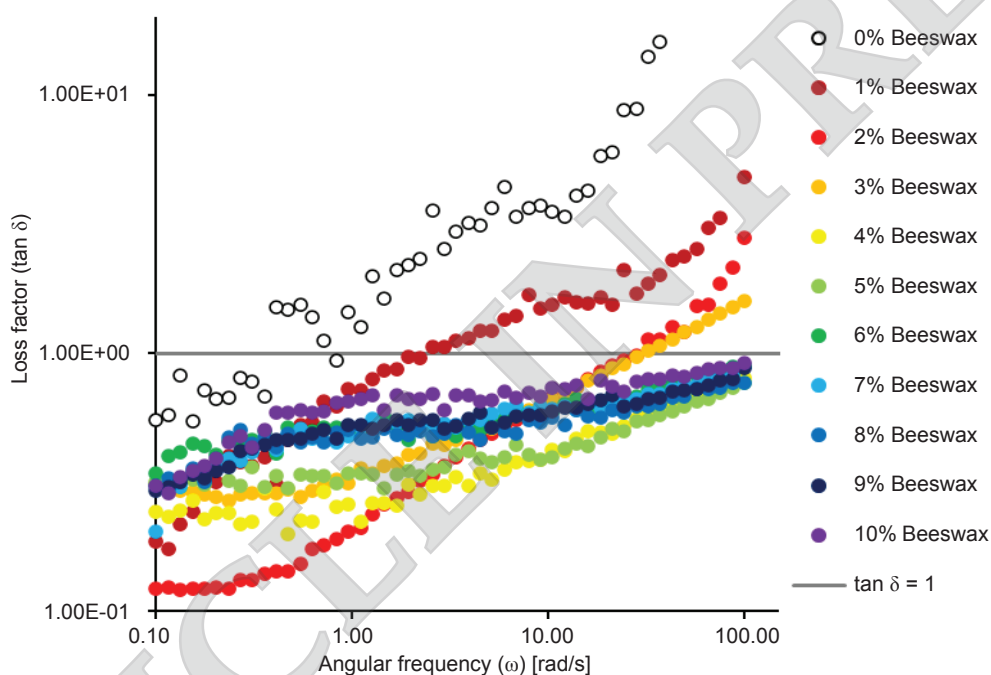


Figure 5. Frequency sweep results of red palm olein oleogels.

TABLE 2. MELTING POINT OF RED PALM OLEIN OLEOGELS

Beeswax concentration (%)	Melting point (°C)
0	<25.00
1	<25.00
2	26.94 ± 1.87 ^a
3	40.61 ± 1.73 ^{bc}
4	39.83 ± 1.49 ^b
5	41.87 ± 2.04 ^{bc}
6	42.90 ± 0.34 ^c
7	45.65 ± 1.87 ^d
8	46.26 ± 1.51 ^d
9	46.82 ± 1.45 ^d
10	47.29 ± 1.56 ^d

Note: Values are the mean ± standard deviation (n=4). Distinct superscript letters indicate significant differences between the melting points of the samples (two-way ANOVA; Duncan's test: $p \leq 0.05$).

2% beeswax had a melting point around room temperatures ($26.94^{\circ}\text{C} \pm 1.87^{\circ}\text{C}$). RPO oleogels with 0% beeswax (control) and 1%-2% beeswax did not form a three-dimensional matrix that firmly trapped the oil molecules. On the other hand, RPO oleogels with 3%-10% beeswax had melting points higher than 25°C , so they were solid at room temperature. This indicated that a minimum of 3% beeswax in the RPO oleogel was in solid form at room temperature.

This melting point of the RPO oleogel was comparable to the results of visual analysis of critical gelation concentrations. RPO oleogel with a melting point above room temperatures (about 25°C) is in solid form at room temperatures, which can be visually identified from the RPO oleogel that was motionless after tube inversion. Oleogel with 3% beeswax had the lowest concentration of beeswax, whose melting point was above 25°C , with a critical gelling concentration of RPO oleogel at 3%. The melting point indicates the temperature limits at which the gel structure can be maintained during heating. These are important for making decisions and controlling the texture, emulsion stability, homogeneity, and other qualities of food during processing.

Solid Fat Content

RPO showed solid fat content (SFC) of $58.89\% \pm 0.46\%$ at 0°C , but it turned to 0% at 20°C . This suggests that at a temperature of 20°C , the RPO was entirely in the liquid phase. In contrast, beeswax had a higher SFC than the RPO samples and all RPO oleogel samples at all temperature levels. The SFC of beeswax was $92.21\% \pm 0.04\%$ at 0°C and reached 0% at 60°C . The SFC value is directly proportional to the melting point of the sample. A higher melting point corresponds to a higher temperature for 0% of SFC.

The melting point profile changed markedly owing to the addition of beeswax to the RPO oleogels. A higher beeswax concentration shifted the SFC chart pattern to the right (Figure 6).

However, the RPO oleogel had a similar pattern of SFC reduction with temperature as the RPO SFC. The increase in the SFC of oleogels resulted from the 3-dimensional structure of the oleogelator, which can trap oils, enhancing the content of saturated fatty acids in the oleogel (Pehlivanoglu *et al.*, 2021). Another study revealed that the SFC of a crude palm oil oleogel with 5% beeswax was higher than that of a cooking oil oleogel with 5% beeswax at all temperatures. It is clearly understandable that CPO has a higher saturated fatty acid content than palm cooking oil, even though it comes from a similar source, that is palm oil (Suriaini *et al.*, 2023). In our experiment, the SFC results were consistent with the results of the melting point analysis during the temperature sweep. When the SFC reached 0%, the temperature was aligned with the melting point of the RPO oleogel. This indicated that the melting point had represented at solid-to-liquid phase transition. A SFC of 0% indicates that the sample is completely liquid.

The temperature range required to achieve a SFC of 0% was close to the melting point analysis results, based on the temperature sweep. RPO oleogel with 2% beeswax had SFC 0% at 30°C , while the melting point of this oleogel was $26.94 \pm 1.87^{\circ}\text{C}$. The SFC 0% was achieved by RPO oleogel with 3%-5% beeswax at 40°C , while RPO oleogel with 6%-10% beeswax reached SFC 0% at 50°C . Therefore, the rheological properties obtained by temperature sweep analysis can replace SFC in determining the transition temperature between the solid and liquid phases at a more specific temperature.

Total Carotenoid

The total carotenoid in RPO and beeswax reached 670.77 ± 1.81 ppm and 94.18 ± 1.69 ppm, respectively. The combination of RPO and beeswax in RPO oleogels at a particular ratio contained various levels of total carotenoids. The results showed that beeswax reduced the total carotenoids contents of the

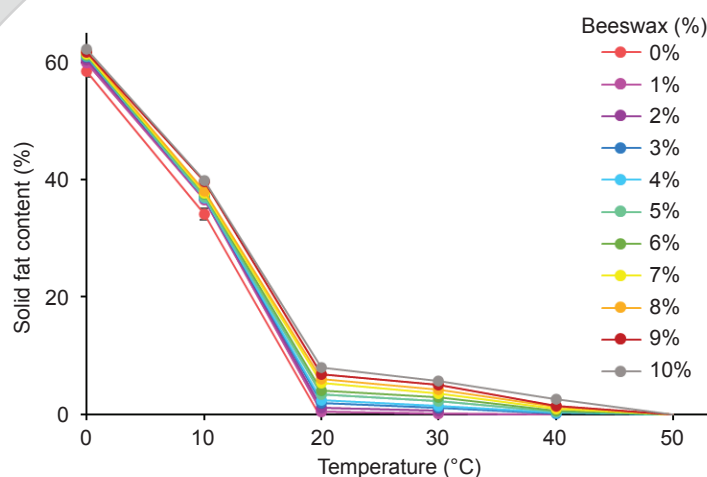


Figure 6. Solid fat content of red palm olein oleogels.

RPO oleogels. In this study, the carotenoid content in the RPO oleogel with 1% beeswax was higher than that in the RPO with 0% beeswax (control). However, the addition of beeswax exceeding 1% significantly increased the total carotenoid content of the RPO oleogels (Figure 7). The increase in the total carotenoid content caused by oleogelation protects specific carotenoid compounds (Puscas *et al.*, 2022). Although increasing the beeswax concentration decreased the total carotenoid levels, adding up to 10% beeswax resulted in high total carotenoid levels of 483.27 ± 2.53 ppm. Thus, RPO oleogels can be used as food ingredients and sources of provitamin A.

Colour Attributes

Colour analysis showed that all the oleogel samples were orange. The beeswax colour was pale yellow and the RPO colour was orange-red. The lightness of the control samples was higher than that of the RPO samples. Noticeably, the

orange colour of the RPO oleogels darkened up to a beeswax concentration of 2%. The lightness of the oleogel containing 3% beeswax was higher than that of the oleogel containing 2% beeswax. At beeswax concentration of 3% onward, the colour pattern (L^* , a^* , b^*) was similar, but they differed compared with 0%-2% beeswax samples (Figure 8), highlighting the impact of the transition phase (liquid at <3% beeswax; solid at $\geq 3\%$ beeswax) on the physical appearance of samples. A higher beeswax concentration resulted in a higher lightness level of the RPO oleogels. This is due to the reduced proportion of RPO. The RPO oleogel, a solid at room temperature, had a paler colour than the liquid RPO oleogel. Therefore, the lightest oleogel sample was the RPO oleogel with 10% beeswax. RPO has the most significant colour effects on oleogel, primarily towing to the carotenoids, which produce a yellow to red colour, due to the presence of conjugated double bonds in the polyene chain (Cvetkovic and Nikolic, 2017; Maoka, 2020).

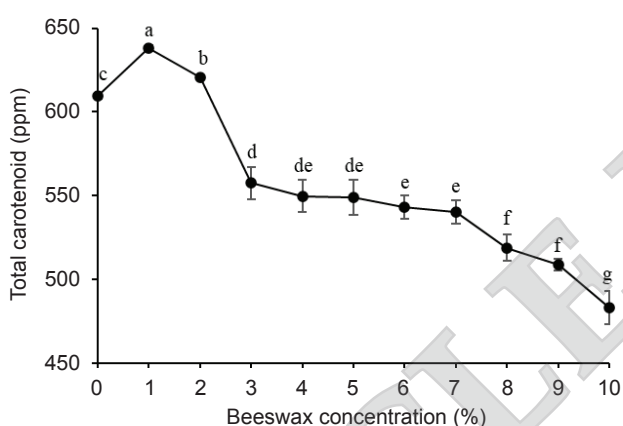


Figure 7. Total carotenoid of red palm olein oleogels. Distinct letters show significant differences between the LVR limit of the samples (two-way ANOVA; Duncan test: $p \leq 0.05$).

CONCLUSION

An RPO oleogel was successfully prepared from RPO by adding beeswax as an oleogelator. We found that a minimum concentration of 3% beeswax is needed to produce a solid oleogel at room temperatures, which is critical in gelling concentration. The oleogelation process induced remarkable changes in the rheological characteristics, SFC, total carotenoids and colour. The results showed that a higher concentration of beeswax produced a more compact needle crystal structure, a wider LVR, a higher yield stress, a higher crossover point, a higher melting point and a higher temperature of SFC 0%. RPO oleogel with 3% beeswax showed a significant parameter

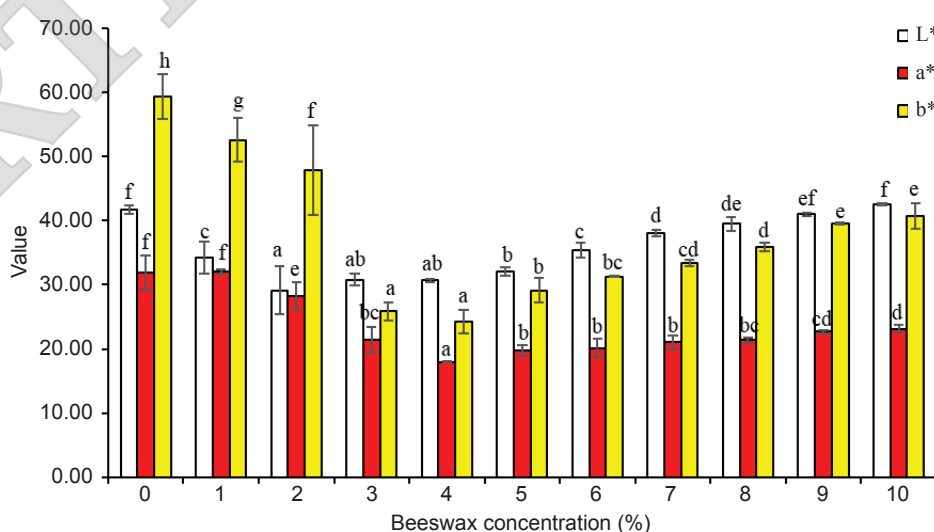


Figure 8. Colour of red palm olein oleogels.

transition, resulting in compact crystal structures that immobilised the RPO, with an LVR limit of $0.02\% \pm 0.04\%$, yield stress of 38.3675 ± 8.1864 Pa, and a melting point of $40.61^\circ\text{C} \pm 1.73^\circ\text{C}$. However, a higher concentration of beeswax yielded a lower total carotenoid content in the RPO oleogels. The decrease in the total carotenoids content correlated with the resulting colour. The lower total carotenoid content also corresponded to a lighter colour of the RPO oleogels, which tended to be yellowish. These RPO oleogels can be used as alternative raw materials as sources of provitamin A for fat-based food products, such as margarine, spreadable products, and shortening.

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REFERENCES

- Alexander, D (2017). *Nature's Machines: An Introduction to Organismal Biomechanics*. Academic Press, Cambridge. p. 99-120.
- American Oil Chemists' Society [AOCS] (2022). *AOCS Official Method Cd 16b-93 Solid Fat Content (SFC) by Low-Resolution Nuclear Magnetic Resonance, Direct Method*.
- Atik, D S; Demirci, M; Toker, O S and Palabiyik, I (2022). Development of a novel rheological method for determining melting properties of gelatin-based gummies. *Int. J. Biol. Macromol.*, 209(1): 385-395. DOI: 10.1016/j.ijbiomac.2022.04.002.
- Barroso, L; Karatay, G and Hubinger, M (2022). Effect of potato starch hydrogel: Glycerol monostearate oleogel ratio on the physico-rheological properties of bigels. *Gels*, 8(11): 694-711. DOI: 10.1016/j.jfca.2010.03.026.
- BSN (Badan Standardisasi Nasional) (2022). *SNI 9098:2022 Minyak Makan Merah*.
- Carazo, A; Macáková, K; Matoušová, K; Krčmová, L K; Protti, M and Mladěnka, P (2021). Vitamin A update: Forms, sources, kinetics, detection, function, deficiency, therapeutic use and toxicity. *Nutrients*, 13(5): 1-36. DOI: 10.3390/nu13051703.
- Chai, X; Zhang, Y; Shi, Y and Liu, Y (2022). Crystallization and structural properties of oleogel-based margarine. *Molecules*, 27(8952): 1-12. DOI: 10.3390/molecules27248952.
- Chasan, R; Rosenberg, D; Kimscha, F; Beeri, R; Golan, D; Ayelet, D; Galili, E and Spiteri, C (2021). Bee products in prehistoric southern levant: Evidence from the lipid organic record. *R. Soc. Open Sci.*, 8: 1-21. DOI: 10.6084/m9.figshare.c.5639082.
- Chen, Y; Gavaliatsis, T; Kuster, S; Stadel, C; Fischer, P and Windhab, E J (2021). Crust treatments to reduce bread staling. *Curr. Res. Nutr. Food Sci.*, 4: 182-190. DOI: 10.1016/j.crfs.2021.03.004.
- Cvetkovic, D. and Nikolic, G (2017). *Carotenoids*. IntechOpen, London. 232 pp.
- Dewi, E K; Mardawati, E and Nurhasanah, S (2023). Color changes evaluation on crude palm oil processing into red palm oil and palm cooking oil as a visual indicator of β -carotene content in oils. *Biomass Biorefin. Bioeconomy*, 1(1): 25-29.
- Dinkgreve, M; Paredes, J; Denn, M M and Bonn, D (2016). On different ways of measuring the yield stress. *J. Non-Newton. Fluid Mech.*, 238: 233-241. DOI: 10.1016/j.jnnfm.2016.11.001.
- Doan, C; To, C M; de Vrieze, M; Lynen, F; Danthine, S; Brown, A; Dewettinck, K and Patel, A R (2017). Chemical profiling of the major components in natural waxes to elucidate their role in liquid oil structuring. *Food Chem.*, 214: 717-725. DOI: 10.1016/j.foodchem.2016.07.123.
- Doan, C D; Tavernier, I; Sintang, M D; Danthine, S; de Walle, D V; Rimaux, T and Dewettinck, K (2017). Crystallization and gelation behavior of low- and high melting waxes in rice bran oil: A case-study on berry wax and sunflower wax. *Food Biophys.*, 12(1): 97-108. DOI: 10.1007/s11483-016-9467-y.
- Espert, M; Hernández, M; Sanz, T and Salvador, A (2022). Rheological properties of emulsion templated oleogels based on xanthan gum and different structuring agents. *Curr. Res. Nutr. Food Sci.*, 5: 564-570. DOI: 10.1016/j.crfs.2022.03.001.
- Eze, S O; Orji, J N; Okechukwu, V U; Omokpariola, D O; Umeh, T C and Oze, N R (2021). Effect of processing method on carotenoid profiles of oils from three varieties of Nigerian palm oil (*Elais guineensis*). *Biophys. Chem.*, 12: 23-31. DOI: 10.4236/jbpc.2021.123003.
- Flöter, E; Wettlaufer, T; Conty, V and Scharfe, M (2021). Oleogels-their applicability and methods of characterization. *Molecules*, 26(6): 1-19. DOI: 10.3390/molecules26061673.
- Gagliardi, M (2020). Rheology of polymer coatings. *Polymer Coatings: Technologies and Applications*

- (Rangappa, S M; Parameswaranpillai, J and Siengchin, S, eds.). 1st edition. CRC Press, Boca Raton. p. 117-136.
- Gómez-Estaca, J; Pintado, T; Jimenez-Colmenero, F and Cofrades, S (2019). Assessment of a healthy oil combination structured in ethyl cellulose and beeswax oleogels as animal fat replacers in low-fat, PUFA-enriched pork burgers. *Food Bioproc. Tech.*, 12: 1068-1081. DOI: 10.1007/s11947-019-02281-3.
- Han, W; Chai, X; Liu, Y; Xu, Y and Tan, C P (2022). Crystal network structure and stability of beeswax-based oleogels with different polyunsaturated fatty acid oils. *Food Chem.*, 381: 1-11. DOI: 10.1016/j.foodchem.2021.131745.
- Hariyadi, P; Purnomo, E H and Kusnandar, F (2019). Aliran dan transportasi fluida. *Landasan Teknik Pangan* (Hariyadi, P ed.). IPB Press, Bogor. p. 91-158.
- Harlen, W; Muchtadi, T and Palupi, N (2018). β -Carotene bioavailability of palm oil emulsion drink in rats (*Rattus norvegicus*) blood plasma and liver. *Int. Food Res. J.*, 25(4): 1349-1356.
- Hasibuan, H; Akram, A; Putri, P and Rangkuti, B T (2019). Pembuatan margarin dan baking shortening dari minyak sawit merah dan aplikasinya dalam produk bakery. *Agriotech*, 38(4): 353-363. DOI: 10.22146/agritech.32162.
- ISO (International Organization for Standardization) (2011). *ISO 17932:2011 Palm Oil - Determination of the Deterioration of Bleachability Index (DOBI) and Carotene Content*.
- ISO (International Organization for Standardization) (2015). *ISO 6721-10:2015 Plastics -Determination of dynamic mechanical properties - Complex shear viscosity using a parallel-plate oscillatory rheometer*.
- Jia, X; Chiu, Q; Zhu, Z; Ding, Q and Gao, P (2022). Influence of fluid flood viscosity on internal flow characteristics of conveying pump. *Front. Nutr.*, 9. DOI: 10.3389/fnut.2022.910589.
- Kamali, E; Sahari, M A; Barzegar, M and Ahmadi, G H (2019). Novel oleogel formulation based on amaranth oil: Physicochemical characterization. *Food Sci Nutr.*, 7(6): 1986-1996. DOI: 10.1002/fsn3.1018.
- Kwon, U H and Chang, Y H (2022). Rheological and physicochemical properties of oleogel with esterified rice flour and its suitability as a fat replacer. *Foods*, 12(2): 10-19. DOI: 10.3390/foods11020242.
- Loganathan, R; Subramaniam, K M; Radhakrishnan, A K; Choo, Y M and Teng, K T (2017). Health-promoting effects of red palm oil: Evidence from animal and human studies. *Nutr. Rev.*, 75(2): 98-113. DOI: 10.1093/nutrit/nuw054.
- Maoka, T (2020). Carotenoids as natural functional pigments. *J. Nat. Med.*, 74(1): 1-16. DOI: 10.1007/s11418-019-01364-x.
- Marliyati, S and Rimbawan, H (2021). Karakteristik fisikokimia dan fungsional minyak sawit merah. *J. Indones. Community Nutr.*, 10(1): 83-94.
- Mezger, T (2021). *Applied Rheology*. 8th edition. Anton Paar GmbH, Graz. 100 pp.
- Morales, E; Iturra, N; Contardo, I; Quilaqueo, M; Franco, D and Rubilar, M (2023). Fat replacers based on oleogelation of beeswax/shellac wax and healthy vegetable oils. *Food Sci. Technol.*, 185: 1-10. DOI: 10.1016/j.lwt.2023.115144.
- Naeli, M H; Milani, J M; Farmani, J and Zargaraan, A (2021). Developing and optimizing low-saturated oleogel shortening based on ethyl cellulose and hydroxypropyl methyl cellulose biopolymers. *Food Chem.*, 369(130963): 1-11. DOI: 10.1016/j.foodchem.2021.130963.
- Nainggolan, M and Sinaga, A G S (2021). Characteristics of fatty acid composition and minor constituents of red palm olein and palm kernel oil combination. *J. Adv. Pharm. Technol. Res.*, 12(1): 22-26. DOI: 10.4103/japtr.JAPTR_91_20.
- Pang, M; Shi, Z; Lei, Z; Ge, Y; Jiang, S and Cao, L (2020). Structure and thermal properties of beeswax-based oleogels with different types of vegetable oil. *Grasas Y. Aceites*, 71(4): 1-11. DOI: 10.3989/gya.0806192.
- Papadaki, A; Kopsahelis, N; Freire, D; Mandala, I and Koutinas, A (2020). Olive oil oleogel formulation using wax esters derived from soybean fatty acid distillate. *Biomolecules*, 10(106): 1-9. DOI: 10.3390/bio10010106.
- Pehlivanoglu, H; Akcicek, A; Can, M A; Karasu, S; Demirci, M and Yilmaz, M T (2021). Effect of oil type and concentration on solid fat contents and rheological properties of watery oleogels'. *Riv. Ital. delle Sostanze Grasse*, 98(3): 177-186.
- Purnama, K O; Setyaningsih, D; Hambali, E and Taniwiryo, D (2020). Processing, characteristics, and potential application of red palm oil - A review. *IJOP*, 3(2): 40-55. DOI: 10.35876/ijop.v3i2.47.
- Puscas, A; Andruta, M; Socaci, S and Fransisc, D (2022). Cold pressed pumpkin seed oil fatty acids,

carotenoids, volatile compounds profiles and infrared fingerprints as affected by storage time and wax based oleogalation. *J. Sci. Food Agric.*, 103(2): 680-691. DOI: 10.1002/jsfa.12180.

Rege, S A; Momin, S A; Wadekar, S D and Bhowmick, D N (2013). Formulation of a functional fat spread stabilised by natural antioxidants and emulsifiers. *Malays. J. Nutr.*, 19(1): 121-130.

Samateh, M; Sagiri, S and John, G (2018). Molecular oleogels: Green approach in structuring vegetable oils (Marangoni, A and Garti, N eds.). *Edible Oleogels*. 2nd edition. AOCS Press, Maryland. p. 415-438.

Sarah, M (2018). Carotenoids preservation during sterilization of palm fruit using microwave irradiation. *J. Eng Appl Sci.*, 13(3): 1009-1014.

SEAIR (2023) *Beeswax Exporters*. <https://www.seair.co.in/beeswax-exporters>, accessed on 11 April 2023.

Sena, B; Dhal, S; Sahu, D; Sarkar, P; Mohanty, B; Jarzebski, M; Wieruszewski, M; Behera, H and Pal, K (2022). Variations in microstructural and physicochemical properties of soy wax/soybean oil-derived oleogels using soy lecithin. *Polymers*, 14(19): 1-16. DOI: 10.3390/polym14193928.

Sivakanthan, S; Fawzia, S; Mundree, S; Madhujith, T and Karim, A (2023). Optimization and characterization of new oleogels developed based on sesame oil and rice bran oil. *Food Hydrocoll.*, 142: 1-27. DOI: 10.1016/j.foodhyd.2023.108839.

Sun, H; Xu, J; Lu, X; Xu, Y; Regenstein, J M; Zhang, Y and Wang, F (2022). Development and characterization of monoglyceride oleogels prepared with crude and refined walnut oil.

Food Sci. Technol., 154: 1-10. DOI: 10.1016/j.lwt.2021.112769.

Suriani, N; Arpi, N; Syamsudin, Y and Supardan, M D (2023). Characteristics of palm oil-based oleogel and its potency as a shortening replacer. *S. Afr. J. Chem. Eng.*, 43: 197-203.

Surman, S L; Penkert, RR; Sealy, R E; Jones B G; Marion, T N; Vogel, P and Hurwitz, J L (2020). Consequences of vitamin A deficiency: Immunoglobulin dysregulation, squamous cell metaplasia, infectious disease, and death. *Int. J. Mol. Sci.*, 21(15): 1-17. DOI: 10.3390/ijms21155570.

Szulc, J; Machnowski, W; Kowalska, S; Jachowicz, A; Ruman, T; Steglińska, A and Gutarowska, B (2020). Beeswax-modified textiles: Method of preparation and assessment of antimicrobial properties. *Polymer*, 12(2): 344-359. DOI: 10.3390/polym12020344.

Wang, X; Wang, S; Nan, Y and Liu, G (2021). Production of margarines rich in unsaturated fatty acids using oxidative-stable vitamin C-loaded oleogel. *J. Oleo Sci.*, 70(8): 1059-1068. DOI: 10.5650/jos.ess20264.

Xu, H; Li T; Zhang, H X; Shi, C H; Cao, J Q and Zhang, X R (2022). The application of oleogels in food products: Classification, preparation, and characterisation. *Acta Aliment.*, 51(4): 462-478. DOI: 10.1556/066.2022.00099.

Zbikowska, A; Onacik-Gür, S; Kowalska, M; Sowiński, M; Szymańska, I; Żbikowska, K; Marciniak-Lukasiak, K and Werpachowski, W (2022). Analysis of stability, rheological and structural properties of oleogels obtained from peanut oil structured with yellow beeswax. *Gels*, 8(7): 1-8. DOI: 10.3390/gels8070448.