CHARACTERISTICS OF SUNFLOWER WAX, CARNAUBA WAX AND BEESWAX IN PALM SUPEROLEIN BLENDED OIL

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ABSTRACT

The aim of the study was to analyse the effect of different percentages (0.0%-3.5%) of sunflower wax (SFW), carnauba wax (CW) and beeswax (BW) in 1:1 (v:v) palm superolein (POOo) and sunflower oil blend (SFO). Selected physicochemical analyses, namely fatty acid composition, crystal structure and shear viscosity were conducted for oleogel or oil system with wax (OSW) selection to be used in a product that is spreadable at low temperature (5°C-10°C) and phase separation does not occur at high temperature (40°C-45°C). Mono-unsaturated and poly-unsaturated as well as saturated fatty acids of all OSW were significantly different (p<0.05). Crystals in OSW with SFW showed needle-like structure and fibrous, while OSW with CW was observed to be spherulitic and OSW with BW was needle-like in shape at 10°C, 25°C, 40°C and 45°C. OSW with 3.5% SFW and 3.5% CW showed dense packing at 45°C; hence, it had the potential to prevent phase separation in the end product at high temperatures. The shear viscosity of all OSW at all temperatures was below 2.0 Pa.s, in which SFW showed the highest value at 5°C, although the value was low. Therefore, based on the experimental parameters, 3.5% CW can be implied as the best structuring agent, followed by 3.5% SFW.

INTRODUCTION

Lipid is the main ingredient used in various types of food products. It imparts texture, aroma, taste and makes food more appealing (Pehlivanoğlu *et al.*, 2018). Most food products that have high lipid content also contain solid fats. These solid fats consist of *trans* fatty acids and/or saturated fatty acids that might have a negative impact on health (de Souza *et al.*, 2015; Wassell *et al.*, 2010). Therefore,

one of the reasons for the utilisation of oleogelation (a gelling agent that traps liquid vegetable oils to form three-dimensional (3D) network system) is due to scientific evidence, which showed that *trans* fatty acids produced from partial hydrogenation are detrimental to health. The partial hydrogenation method uses liquid vegetable oil by converting liquid oil into a solid form with the desired hardness. As *trans* fatty acids are harmful to health, the GRAS status (generally recognised as safe) for *trans* fatty acids was revoked by the Food and Drug Administration (FDA), the United States on 16 June 2015. The use of *trans* fatty acids in food products was fully banned in the United States starting June 2018 (FDA, 2018).

Oleogelation is also proposed to replace the use of saturated fatty acids to improve the nutritional properties of food products as it only utilises unsaturated fatty acids and gels. This oleogelation

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method does not change the saturation level of fatty acids found in food products (Öğütcü and Yilmaz, 2014). The use of unsaturated fatty acids without gel is technically challenging, especially in the structuring of food products. For example, liquid vegetable oils that contain many unsaturated fatty acids give unsatisfactory performance when used for cake-making and related purposes due to their low viscosity (Kim *et al.*, 2017).

To replace these *trans* and saturated fatty acids, the oleogelation method that uses gels to produce oleogel was reported to physically replace the function and texture of solid fats by trapping liquid vegetable oils in their network structure (Daniel and Rajasekharan, 2003; Hwang *et al.,* 2012; Singh *et al.,* 2017). The oleogel produced has solid / gel-like properties (Co and Marangoni, 2012). Nevertheless, this type of oleogel will still have a high content of unsaturated fatty acids and low saturated fatty acids. Furthermore, the oleogel does not contain water, possesses viscoelasticity and can undergo a process of thermal reversal (Gallego *et al.,* 2013; Hughes *et al.,* 2009).

The incorporation of oleogel into various products has made oleogelation an important structuring method that was widely researched and applied over the past two decades (Co and Marangoni, 2012; Zetzl and Marangoni, 2014). For example, the cosmetics and pharmaceutical industries have used this oleogelation method for a long time to produce a desirable stable product by using liquid organic solvents such as benzene and hexane (Balasubramaniam *et al.,* 2014; Kumar and Katare, 2005; Vintiloiu and Lerouex, 2008). The term organogelation is usually used for organic solvents, while the term oleogelation is used when this organic solvent is replaced with liquid vegetable oil. The mechanism of oleogelation depends on various factors, amongst which are the composition of a gel material and its interaction with the lipid phase. The most commonly used method for liquid vegetable oil structuring is the addition of a gel material (one or more ingredients) directly into the liquid vegetable oil. One of the gel ingredients is food-grade wax. Wax is commonly used in small quantities to produce oleogel (Doan *et al.,* 2018; Hughes *et al.,* 2009; Patel *et al.,* 2013; Sánchez *et al.,* 2011; Stortz *et al.,* 2012). Waxes are categorised as non-lipid monomeric liners. In addition to waxes, other gel materials that can be used are polymeric coatings (ethyl cellulose), and lipid monomeric coatings (fatty acids, phospholipids, monoglycerides and ceramides) and non-lipid monomeric coatings (sorbitol and γ-oryzanol phytosterols other than wax) (Rogers *et al.,* 2014).

This article will discuss the fatty acid composition (FAC), crystal structure and shear viscosity of blended oleogel or oil system with wax (OSW), containing 50% of palm superolein (POOo) and 50% of sunflower oil (SFO) with various percentages of sunflower wax (SFW), carnauba wax (CW) and beeswax (BW) to obtain OSW that can be spreadable at low temperature (5°C-10°C), but the oleogel will not experience phase separation at high temperature (40°C-45°C). This characteristic has not yet been researched or published. The rationale for the selection of 50% of POOo and 50% of SFO base oil was discussed in the paper by Norazura *et al*. (2021). In this article, the term OSW will be used to replace oleogel.

MATERIALS AND METHODS

Material

POOo (IV 62) was bought from Mewah Oils Sdn. Bhd., while sunflower oil (SFO) (Naturel brand) was from a local supermarket in Selangor. SFW was bought from Hufaqa Sepakat Enterprise, Selangor, Malaysia. Meanwhile CW and BW were from Anizz Legacy Resources, Selangor, Malaysia.

Method

Preparation of OSW. As shown in *Table 1*, three types of waxes, namely SFW, CW and BW at different percentages (0.0%-3.5%) were blended with 1:1 (v:v) palm superolein and sunflower oil. Then, OSW was heated to 90°C and stirred to dilute the waxes.

TABLE 1. EXPERIMENTAL DESIGN OF OIL SYSTEM WITH SUNFLOWER WAX, CARNAUBA WAX AND BEESWAX

Oil	Type of wax	Percentage of wax
Superolein and sunflower wax (1:1)	Sunflower wax (SFW)	0.0%
		0.5%
		1.0%
		1.5%
		2.0%
		2.5%
		3.0%
		3.5%
	Carnauba wax (CB)	0.0%
		0.5%
		1.0%
		1.5%
		2.0%
		2.5%
		3.0%
		3.5%
	Beeswax (BW)	0.0%
		0.5%
		1.0%
		1.5%
		2.0%
		2.5%
		3.0%
		3.5%

Fatty acid composition **(FAC)**. FAC of oil system with wax was analysed according to MPOB Test Method (MPOB, 2005) based on fatty acid methyl esters (FAME) by using gas chromatography. Then, samples of 0.1 g were weighed and dissolved in hexane (1.8 mL). The solution of sample was mixed by using a vortex mixer. Sodium methoxide solution (100 μL) was added to the samples, followed by water. Mixing was performed in between each process. The sample solution was left for 1 hr to separate. The clear supernatant was transferred into another vial with added sodium sulphate anhydrous. It was kept for 15 min before a complete clear supernatant of 1.5 mL was injected into GC (Agilent Technologies, Model 7890B) that has a flame ionisation detector (FID). The column was set at 185°C while the injector and detector temperatures were set at 230°C. Helium gas (carrier) with a flow rate of 1 mL/min was used. Identification of peaks was done by comparing retention times with FAME standards and quantified by using the peak area normalisation method.

Crystal structure. The crystal microstructure of OSW was determined by using a polarised light microscope (PLM) as described by Chai *et al.* (2018) with slight modification. A Leica DMLP polarised light microscope (Wetzlar, Germany) equipped with a Linkam THMS 600 temperature controller stage and a JVC 3-CCD colour video camera was used. The sample was first heated to 90°C for at least 60 min to erase crystal memory. A small droplet (about 15 μL) of the melted sample was placed on a glass slide and covered with a glass slip. The sample was measured at 10°C, 25°C, 40°C and 45°C. The temperature was thermostatically controlled by a Linkam TP 94 multiramp temperature programmer and LNP automatic cooling system (Linkam, Tadworth, Surrey, United Kingdom). Liquid nitrogen was used as the coolant. The Photomicrograph of the crystal was taken at 10× magnification.

Rheology (shear viscosity). Shear viscosity of OSW was measured by using modular advanced rheometer (Haake, Mars). OSW (17 g) were placed in a cylindrical cup (diameter of 27.206 mm) with rotor (diameter of 25.082 mm). Sample was tested at temperature of 5°C, 10°C, 25°C, 40°C and 45°C. Prior to this, the OSW was stored at 5°C, 10°C, 25°C, 40°C and 45°C for 24 hr. The shear viscosity was measured at 100, 200, 300, 400 and 500/s.

Statistical analysis. Results were analysed with Minitab Version 16.0 (Pennsylvania, United States) by using one-way ANOVA. Significance differences ($p \le 0.05$) amongst the samples were analysed by using Tukey's post-hoc test. All tests were carried out in triplicate.

RESULTS AND DISCUSSION

The Effect of Waxes on FAC of OSW

FAC of POOo and SFO as well as oil system containing 50.00% POOo and 50.00% SFO with 0.00%-3.50% SFW, CW and BW, were shown in *Table 2*. POOo contained 58.46% unsaturated fatty acids (USAFA, combination of monounsaturated and polyunsaturated fatty acids) and 41.54% of saturated fatty acids (SAFA). Meanwhile SFO had 91.24% of USAFA and 8.76% of SAFA. POOo was high in oleic acid (45.09%) and palmitic acid (36.01%), while SFO was high in linoleic acid (55.96%) and oleic acid (34.68%). FAC of POOo at the range of 45.26%-46.26% of oleic acid and 34.9%-36.13% of palmitic acid were reported by Abdel-Razek *et al.* (2017), Ng *et al.* (2014) and Romano *et al.* (2012).

An oil system containing 50.00% POOo and 50.00% SFO without wax had 73.75% of USAFA and 26.25% of SAFA. The oil system without wax was also high in oleic, linoleic and palmitic acids. The oil demonstrated increment in USAFA and decrement in SAFA as compared to 100% POOo. The OSW with SFW, CW and BW at all percentages had a similar FAC trend, in which they were high in oleic, linoleic and palmitic acids. Within OSW with SFW, oleic acid of 2.50% SFW and 3.50% SFW were significantly different ($p < 0.05$) from other experimental percentages (0.50%, 1.00%, 1.50%, 2.00% and 3.00%) while for OSW with CW and BW, 3.50% of wax was significantly different ($p < 0.05$) from other experimental percentages (0.50%, 1.00%, 1.50%, 2.00%, 2.50% and 3.00%) within their own system. For linoleic acid, OSW with 3.00% SFW had significantly different values ($p < 0.05$) as compared to other experimental percentages (0.50%, 1.00%, 1.50%, 2.00%, 2.50% and 3.50%) while for OSW with CW and BW, 3.50% of wax was significantly different ($p < 0.05$) from other experimental percentages (0.50%, 1.00%, 1.50%, 2.00%, 2.50% and 3.00%) within their own system. For palmitic acid, OSW with 2.00% and 3.00% SFW, OSW with 3.00% and 3.50% CW and OSW with 3.50% BW had significantly different values ($p < 0.05$) within their own systems. Although FAC values of the waxes were significantly different ($p < 0.05$), their range was small, and thus, their saturation level was comparable due to not much difference observed for the overall composition range of their respective FAC. OSW with SFW at all percentage levels had oleic acid in the range of 37.44%-41.51%, CW in the range of 35.53%-37.28% and BW in the range of 36.46%-39.65%. Linoleic acid of OSW with SFW, CW and BW of all experimental percentages (0.50%-3.50%) was in the range of 32.84%-36.30%, 35.00%-39.91% and 34.69%-36.79%, respectively. This was in line with the findings

by Jang *et al.* (2015) which showed quite a similar FAC value for all fatty acids when 3.00% and 6.00% of candelilla wax were added to canola oil as compared to the FAC value of 100.00% canola oil. Notwithstanding this, the fatty acid values of OSW were quite close to the value of the oil system without wax.

USAFA of OSW ranged from 74.88%-76.02% for SFW, 74.75%-75.94% for CW and 75.39%-76.10% for BW. SAFA of OSW with SFW, CW and BW were in the range of 23.93%-26.33%, 24.02%-26.39% and 23.41%-27.41%, respectively. OSW of all waxes was statistically significant in terms of MONO, POLY and SAFA. However, no trend was observed with different percentages of wax as the values of USAFA and SAFA did not vary much, and thus, would not affect the saturation level.

The Effect of Waxes on Crystal Structure of OSW

Crystal structure of OSW with SFW, CW and BW at 0.5%-3.5% for 10°C, 25°C, 40°C and 45°C are shown in *Figures 1, 2* and *3*. Crystal structure at 5°C could not be determined in the reaction of OSW with SFW, CW and BW for 0.5%-3.5% due to water condensation. Theoretically, there will be no crystal for the oil system without wax at 10°C and above as both of the oil used were liquid oil. Moreover, more than 70.0% of their FAC was USAFA. Crystal in OSW with SFW showed needle-like structure and fibrous similar to the findings by Patel *et al.* (2014). The fibrous morphology of the system reflected the high content of wax esters in SFW, which was a major component that contributed to the good gelation (Blake *et al.*, 2014). The network of SFW crystals experienced significant changes when the percentage of SFW was increased from 0.5% to 3.5% for all experimental temperatures. At 10°C, OSW with SFW showed a denser structure when the wax percentage was increased from 0.5% to 3.5%, and this could affect the end product which required good spreadability at that temperature. At 25°C, a similar result as at 10°C was observed when the wax percentage increased. However, the packing was less dense than at 10°C. This trend was similar to the results by Sandoval *et al.* (2020), which had 2.0%, 3.5% and 5.0% of SFW at room temperature. At 40°C and 45°C, crystals in the OSW with SFW were still dense except for 0.5% and 1.0% SFW as well as 0.5%, 1.0% and 1.5% SFW, respectively. There was an increase in birefringence at a high concentration of SFW and crystals were observed to be aggregated. The high percentage of SFW in the emulsion may produce nucleation of crystal and domination of crystal growth, which resulted in finer crystals (Sandoval *et al.*, 2020). Due to the morphology and arrangement of the SFW network, an increase in hardness might be found

in OSW which had a higher percentage of SFW, especially at 10°C. In addition, 3.5% SFW showed dense packing. It was interesting to observe that 2.5%, 3.0% and 3.5% SFW were still in dense packing at 45°C; hence, could better retain the base oil from oiling out. Notwithstanding this, OSW with 2.0%-3.5% SFW was observed to be dense in packing and could affect the end product which required good spreadability at that temperature.

The crystal structure of OSW with CW was observed to be spherulitic. A similar finding was reported by Öğütcü and Yılmaz (2015) with 7.0% and 10.0% addition of CW to hazelnut oil. This was also supported by Dassanayake *et al.* (2009) for CW in olive oil. The crystal structure of OSW with CW at 10°C was almost evenly spread for all wax percentages. Finer crystals were observed at a low percentage of CW. At 3.5% CW, agglomeration of crystals was observed. At 25°C, OSW with 3.5% CW was observed to have denser packing compared to other wax percentages. At 3.0% CW, the crystal structures were coarse. OSW with 0.5% and 1.0% CW was liquefied with very minor crystals present at 25°C. At 40°C and 45°C, the crystal structure became denser when it reached 3.0% CW, which was similar to the trend of OSW with CW at 25°C. However, the crystals became coarser and lumpy at 3.5% CW at 40°C and 45°C. Crystal in OSW of 2.5% CW at 45°C was more homogenised than 3.0% CW at similar temperatures. OSW with 3.0% and 3.5% CW were observed to be less dense as compared to SFW at similar percentages at 45°C, and thus, oiling out might occur more (in volume) compared to 3.5% SFW. However, wax structure, especially for OSW with 3.5% CW, was observed to trap base oil, and thus, might hinder the base oil from excessive oiling out. OSW with 3.5% CW also showed dense packing but lesser than OSW with 3.5% SFW at 10°C; hence, it was anticipated to be more spreadable as compared to OSW with 3.5% SFW but with a coarser texture.

The crystal structure of OSW with BW at all percentages and experimental temperatures was needle-like in shape, which was similarly reported by Moghtadaei *et al.* (2018) and Martin *et al.* (2016). At 10°C, 25°C, 40°C and 45°C, less crystal was observed for OSW at 0.5%-1.5%. OSW with a higher percentage of wax yielded a denser system but was influenced by temperature. However, for 3.5% BW at 40°C, the crystal became clumpy. Notwithstanding this, this denser crystal system was similar to the finding by Martin *et al.* (2017) who observed a denser packing for 2.0%-8.0% BW in high oleic sunflower oil. The densest crystal was observed at 3.5% BW at 10°C. In view that the crystal structure of OSW with 3.5% BW was very loose in packing at 45°C, the OSW might oil out more and faster than 3.5% SFW and 3.5% CW.

TABLE 2. FATTY ACID COMPOSITION OF OIL SYSTEM (50% PALM SUPEROLEIN & 50% SUNFLOWER OIL) WITHOUT AND WITH SUNFLOWER WAX, CARNUBA WAX AND BEESWAX AT VARIOUS PERCENTAGE

Sample	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	OTHERS	MONO	POLY	SAT.
POOo 100%	0.44±0.02[a]	1.08±0.00[a]	36.01±0.21[a]	0.32±0.05[a]	3.76±0.06[d]	45.09±0.31[a]	12.69±0.30[m]	0.36±0.12[defg]	0.24±0.07[efg]	N/D	0.24±0.00[a]	45.41±0.28[a]	13.05±0.38[h]	41.54±0.10[a]
SFO 100%	N/D	0.07±0.01[e]	5.55±0.20[l]	N/D	3.06±0.04[f]	34.68±0.33[m]	55.96±0.10[a]	0.60±0.01[cdeg]	0.10±0.00[cdeg]	N/D	0.00±0.00[a]	34.68±0.04[l]	56.56±0.26[a]	8.76±0.22[l]
SFW 0.0%	0.17±0.01[bcd]	0.59±0.02[b]	21.45±0.08[bc]	0.22±0.02[b]	3.83±0.07[cd]	39.88±0.11[ef]	33.40±0.05[kl]	0.26±0.08[efg]	0.22±0.08[efg]	N/D	0.00±0.00[a]	40.10±0.13[cde]	33.65±0.06[g]	26.25±0.12[c]
SFW 0.5%	0.17±0.03[bcd]	0.54±0.02[bcd]	21.39±0.05[bcd]	N/D	2.00±0.01[h]	41.48±0.19[c]	33.42±0.08[kl]	0.88±0.15[bcd]	0.12±0.11[bcd]	N/D	0.00±0.01[a]	41.48±0.19[b]	34.30±0.21[g]	24.22±0.09[ghij]
SFW 1.0%	0.16±0.01[bcd]	0.53±0.20[cd]	21.12±0.07[cd]	N/D	2.90±0.07[g]	40.63±0.00[c]	34.38±0.05[ji]	0.14±0.01[fg]	0.11±0.01[fg]	N/D	0.04±0.03[a]	40.63±0.00[c]	34.52±0.04[g]	24.81±0.07[def]
SFW 1.5%	0.19±0.00[bcd]	0.55±0.01[bcd]	21.25±0.02[bcd]	N/D	2.99±0.02[g]	40.52±0.04[cd]	34.25±0.06[k]	0.11±0.01[g]	0.14±0.04[g]	N/D	0.00±0.00[a]	40.52±0.04[cd]	34.36±0.07[g]	25.12±0.04[de]
SFW 2.0%	0.22±0.02[bcd]	0.57±0.03[bc]	20.35±0.10[efg]	N/D	2.94±0.03[g]	39.95±0.20[de]	35.49±0.43[efgh]	0.36±0.10[efg]	0.10±0.06[fg]	N/D	0.01±0.02[a]	39.95±0.20[de]	35.86±0.32[f]	24.19±0.12[ghij]
SFW 2.5%	0.23±0.01[bcd]	0.55±0.00[bcd]	21.56±0.06[b]	N/D	2.05±0.00[h]	41.51±0.01[b]	32.84±0.00[l]	0.96±0.01[abc]	0.26±0.01[abc]	N/D	0.04±0.05[a]	41.51±0.01[b]	33.80±0.01[g]	24.65±0.05[efg]
SFW 3.0%	0.25±0.08[bc]	0.53±0.02[cd]	20.15±0.13[efgh]	N/D	2.90±0.03[g]	39.27±0.12[fgh]	36.30±0.13[de]	0.45±0.05[defg]	0.10±0.02[defg]	N/D	0.05±0.08[a]	39.27±0.12[fg]	36.75±0.18[def]	23.93±0.02[k]
SFW 3.5%	0.13±0.00[d]	0.54±0.01[bcd]	21.30±0.07[bcd]	N/D	4.09±0.01[b]	37.44±0.05[l]	35.04±0.10[ghij]	1.19±0.19[ab]	0.27±0.01[ab]	N/D	0.00±0.00[a]	37.44±0.05[m]	36.23±0.11[ef]	26.33±0.06[c]
CW 0.0%	0.17±0.01[bcd]	0.59±0.02[b]	21.45±0.08[bc]	0.22±0.02[b]	3.83±0.07[cd]	39.88±0.11[ef]	33.40±0.05[kl]	0.26±0.08[efg]	0.22±0.08[efg]	N/D	0.00±0.00[a]	40.10±0.13[cde]	33.65±0.06[g]	26.25±0.12[c]
CW 0.5%	0.14±0.01[cd]	0.53±0.01[cd]	20.37±0.02[efg]	N/D	2.80±0.19[g]	35.98±0.1[kl]	39.66±0.30[b]	0.30±0.17[efg]	0.17±0.03[efg]	N/D	0.04±0.07[a]	35.98±0.09[ghijk]	39.96±0.13[b]	24.02±0.10[ji]
CW 1.0%	0.16±0.02[bcd]	0.53±0.01[cd]	20.39±0.07[ef]	N/D	2.92±0.06[g]	35.58±0.33[l]	39.39±0.31[b]	0.62±0.13[cdef]	0.33±0.05[cdef]	N/D	0.09±0.08[a]	35.58±0.33[k]	40.01±0.41[b]	24.33±0.19[ghi]
CW 1.5%	0.18±0.01[bcd]	0.53±0.02[cd]	20.39±0.17[ef]	N/D	2.89±0.03[g]	35.76±0.20[l]	39.77±0.55[b]	0.37±0.17[efg]	0.11±0.04[efg]	N/D	0.00±0.00[a]	35.76±0.17[k]	40.14±0.39[b]	24.10±0.23[hij]
CW 2.0%	0.18±0.01[bcd]	0.53±0.02[cd]	20.38±0.11[efg]	N/D	2.89±0.02[g]	35.53±0.20[l]	39.91±0.08[b]	0.50±0.20[cdeg]	0.06±0.01[cdeg]	N/D	0.02±0.03[a]	35.53±0.16[k]	40.41±0.28[b]	24.05±0.10[ji]
CW 2.5%	0.18±0.06[bcd]	0.53±0.00[cd]	20.45±0.06[e]	N/D	2.95±0.02[g]	35.83±0.00[kl]	39.35±0.01[b]	0.51±0.16[cdeg]	0.20±0.12[cdeg]	N/D	0.00±0.01[a]	35.83±0.03[k]	39.86±0.18[b]	24.30±0.15[fghij]
CW 3.0%	0.26±0.13[b]	0.54±0.03[bc]	21.37±0.12[bcd]	N/D	2.95±0.00[g]	36.60±0.10[j]	38.02±0.53[c]	0.12±0.01[g]	0.08±0.02[g]	N/D	0.05±0.09[a]	36.60±0.15[j]	38.14±0.53[c]	25.20±0.31[d]
CW 3.5%	0.13±0.00[d]	0.57±0.00[bc]	21.32±0.03[bcd]	N/D	4.08±0.06[bc]	37.28±0.13[c]	35.00±0.32[ghij]	1.18±0.07[ab]	0.28±0.05[ab]	0.12±0.02[a]	0.02±0.04[a]	37.41±0.11[h]	36.18±0.24[ef]	26.39±0.12[c]
BW 0.0%	0.17±0.01[bcd]	0.59±0.02[b]	21.45±0.08[bc]	0.22±0.02[b]	3.83±0.07[cd]	39.88±0.11[ef]	33.40±0.05[kl]	0.26±0.08[efg]	0.22±0.08[efg]	N/D	0.00±0.00[a]	40.10±0.13[cde]	33.65±0.06[g]	26.25±0.12[c]
BW 0.5%	0.15±0.01[d]	0.51±0.01[d]	19.63±0.13[h]	N/D	2.98±0.10[g]	39.10±0.16[gh]	36.73±0.20[d]	0.65±0.06[cde]	0.13±0.05[cde]	N/D	0.11±0.10[a]	39.10±0.16[g]	37.36±0.25[cd]	23.41±0.04[k]
BW 1.0%	0.15±0.01[cd]	0.52±0.01[cd]	20.01±0.04[fgh]	N/D	3.08±0.10[d]	39.07±0.30[gh]	36.79±0.10[d]	0.25±0.14[de]	0.06±0.02[de]	N/D	0.08±0.10[a]	39.07±0.27[g]	37.04±0.21[de]	23.82±0.05[k]
BW 1.5%	0.15±0.02[bcd]	0.52±0.01[cd]	19.97±0.35[gh]	N/D	3.35±0.06[e]	39.65±0.49[efg]	35.76±0.50[efg]	0.48±0.32[cdeg]	0.12±0.02[cdeg]	N/D	0.01±0.01[a]	39.65±0.49[ef]	36.24±0.82[ef]	24.11±0.34[hij]
BW 2.0%	0.16±0.00[bcd]	0.53±0.01[cd]	20.27±0.00[efg]	N/D	3.59±0.09[de]	39.60±0.18[efg]	35.23±0.10[fghi]	0.54±0.10[cdeg]	0.08±0.02[cdeg]	N/D	0.00±0.00[a]	39.60±0.18[ef]	35.77±0.25[f]	24.63±0.06[efgh]
BW 2.5%	0.16±0.01[bcd]	0.53±0.01[cd]	20.18±0.15[fgh]	N/D	3.61±0.12[de]	39.08±0.19[gh]	36.15±0.50[def]	0.23±0.05[efg]	0.08±0.02[efg]	N/D	0.00±0.00[a]	39.08±0.19[g]	36.38±0.42[def]	24.54±0.24[fghi]
BW 3.0%	0.15±0.01[bcd]	0.52±0.00[cd]	19.98±0.15[fgh]	N/D	3.73±0.07[d]	38.82±0.21[g]	36.05±0.46[def]	0.53±0.02[cdeg]	0.22±0.06[cdeg]	N/D	0.00±0.00[a]	38.82±0.21[g]	36.58±0.48[def]	24.60±0.26[efgh]
BW 3.5%	0.13±0.01[d]	0.59±0.06[b]	20.98±0.18[d]	N/D	5.29±0.23[a]	36.46±0.32[k]	34.69±0.42[hij]	1.43±0.45[a]	0.42±0.26[a]	N/D	0.00±0.00[a]	36.46±0.32[k]	36.13±0.11[ef]	27.41±0.31[b]

Note: Means within each column bearing different superscript letters are significantly different ($p<0.05$).
N/D - not detected; POOo - palm superolein; SFO - sunflower oil; SFO - sunflower oil; 12:0 - lauric acid; 14:0 - myristic acid; 16:0 - palmitic acid; 16:1 - palmitoleic acid; 18:0 - stearic acid; 18:1 - oleic acid; 18:2 - linoleic acid; 18:3 - linolenic acid; 20:0 - arachidic acid; 20:1 - eicosenoic acid; MONO - monounsaturated fatty acid; POLY - polyunsaturated fatty acid; SAT. - saturated fatty acid.
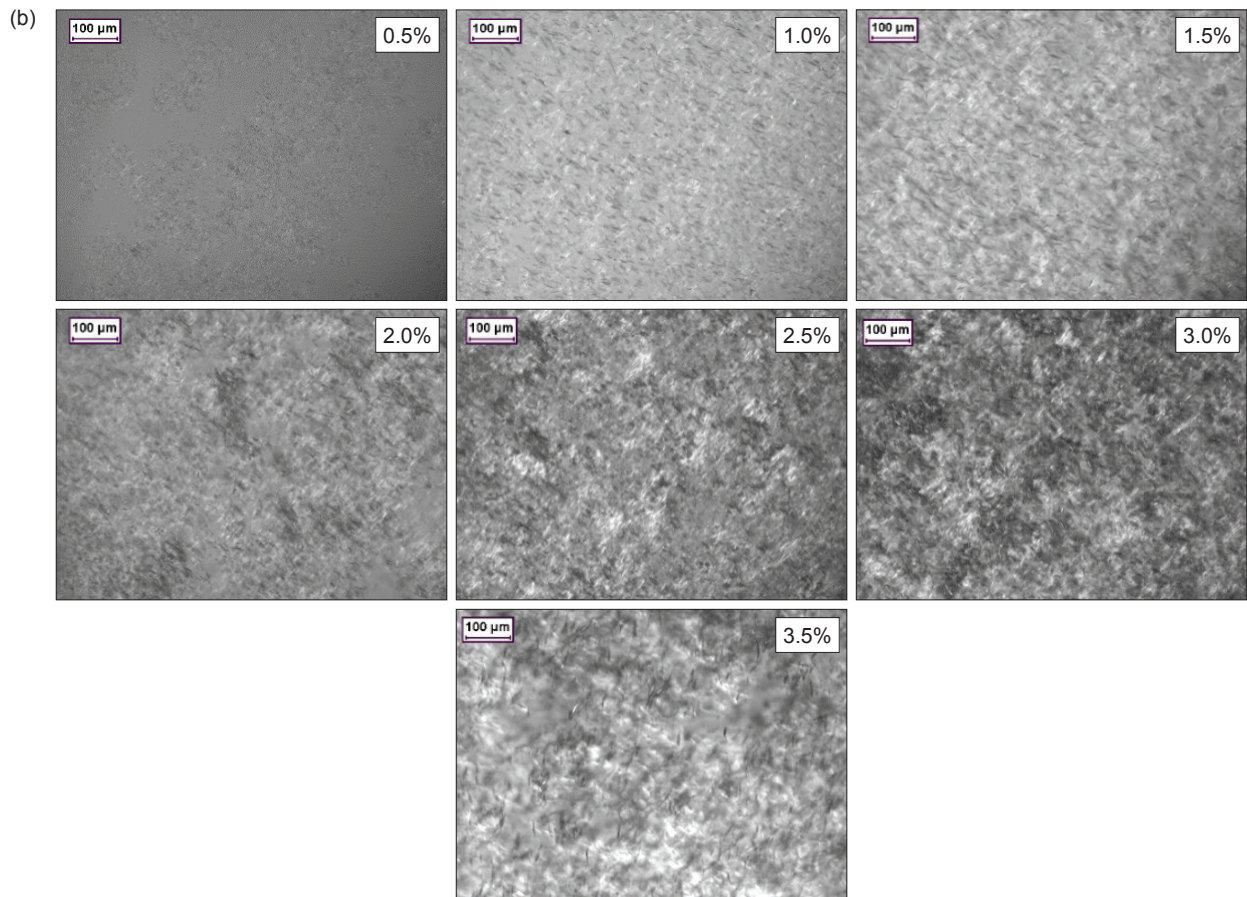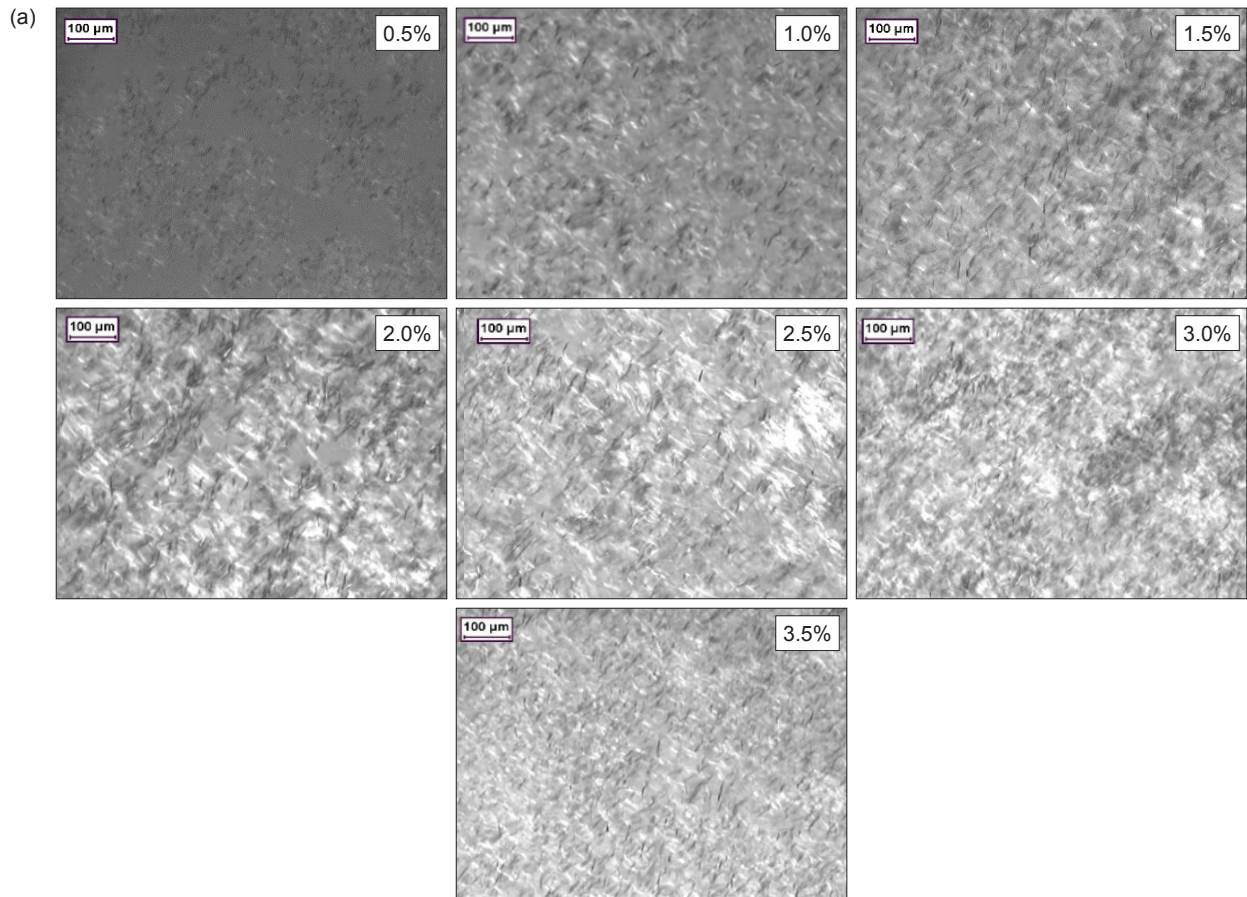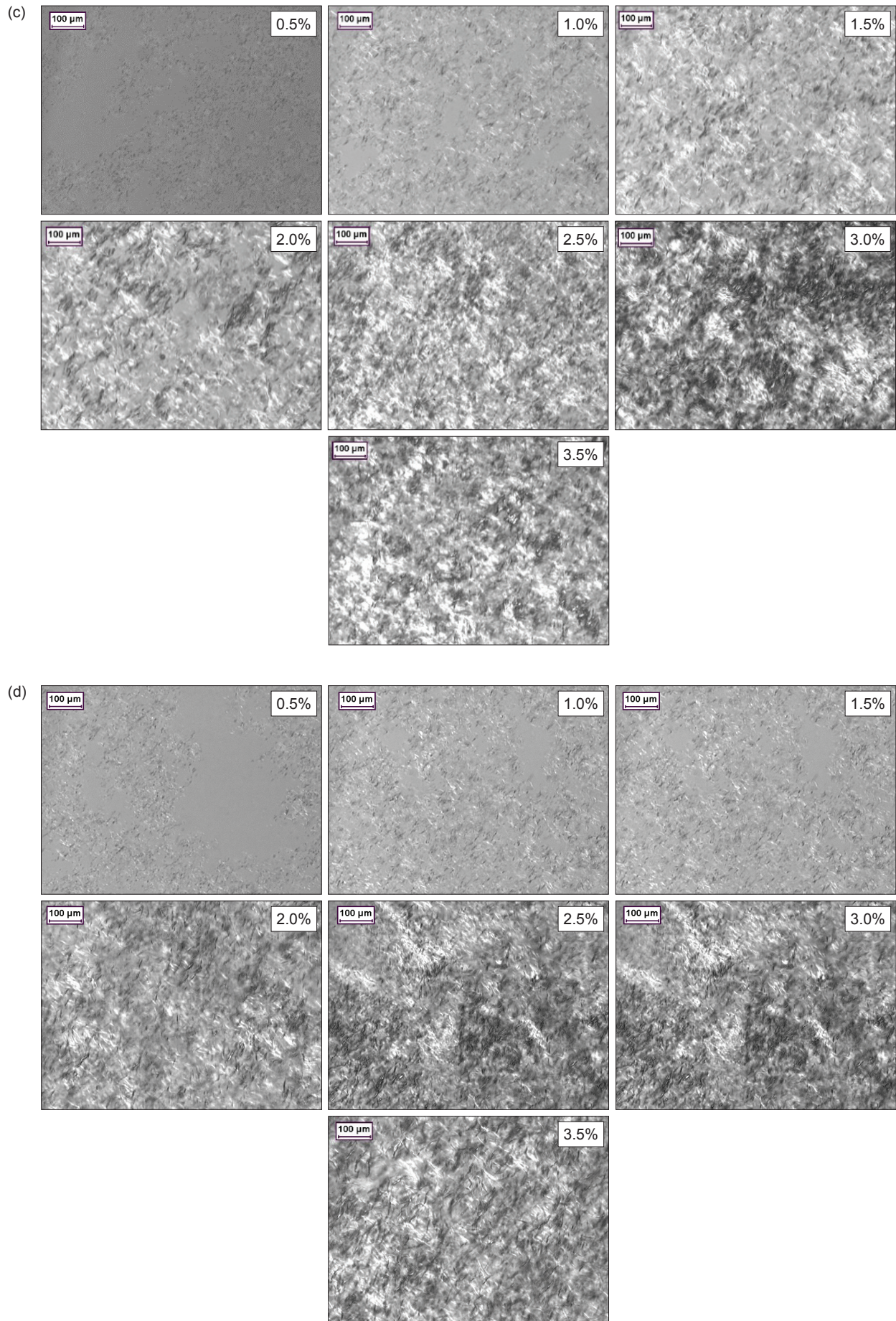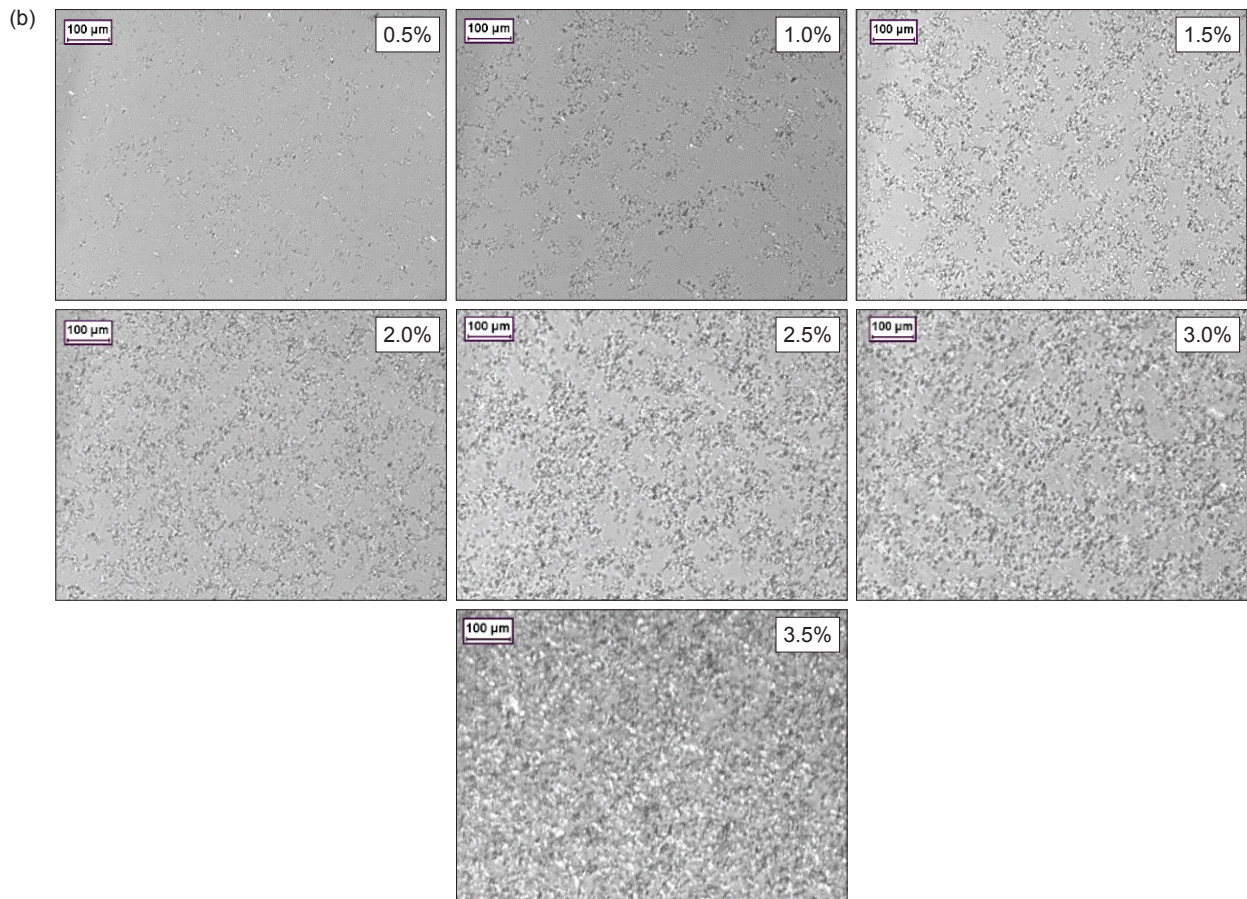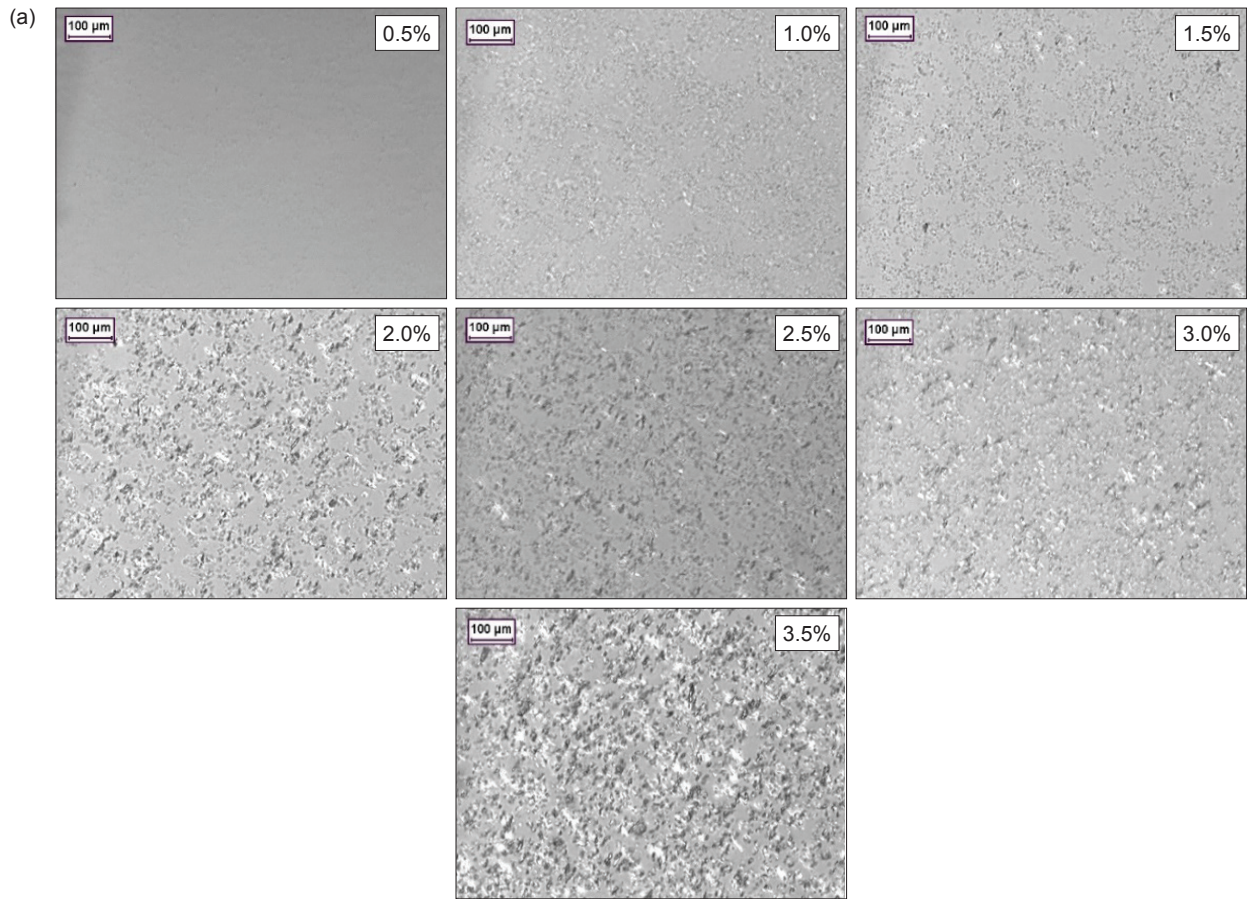
Figure 1. Polarised light micrographs (10x lens) of oil system with 0.5%-3.5% sunflower wax at (a) 10℃, (b) 25℃, (c) 40℃ and (d) 45℃.
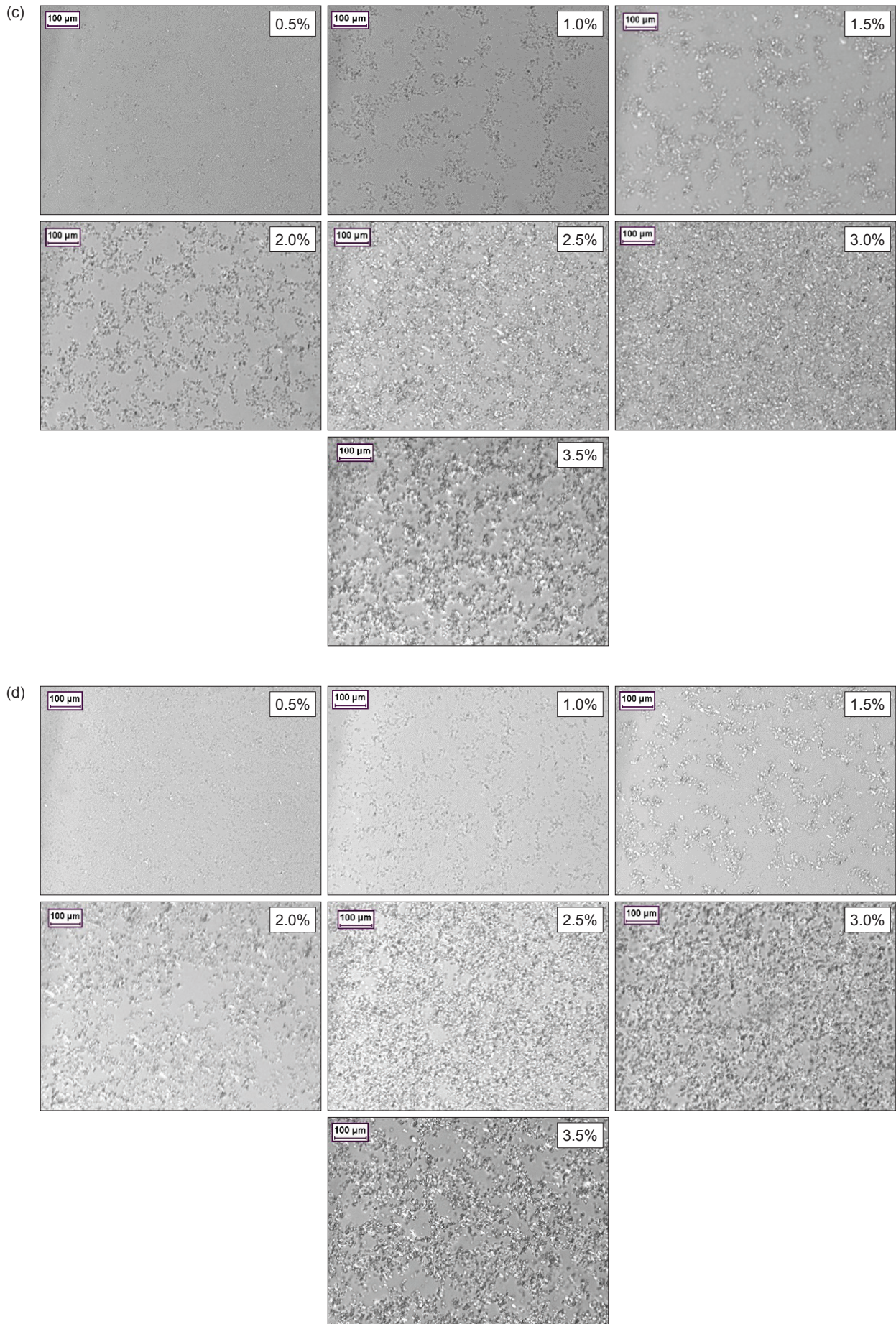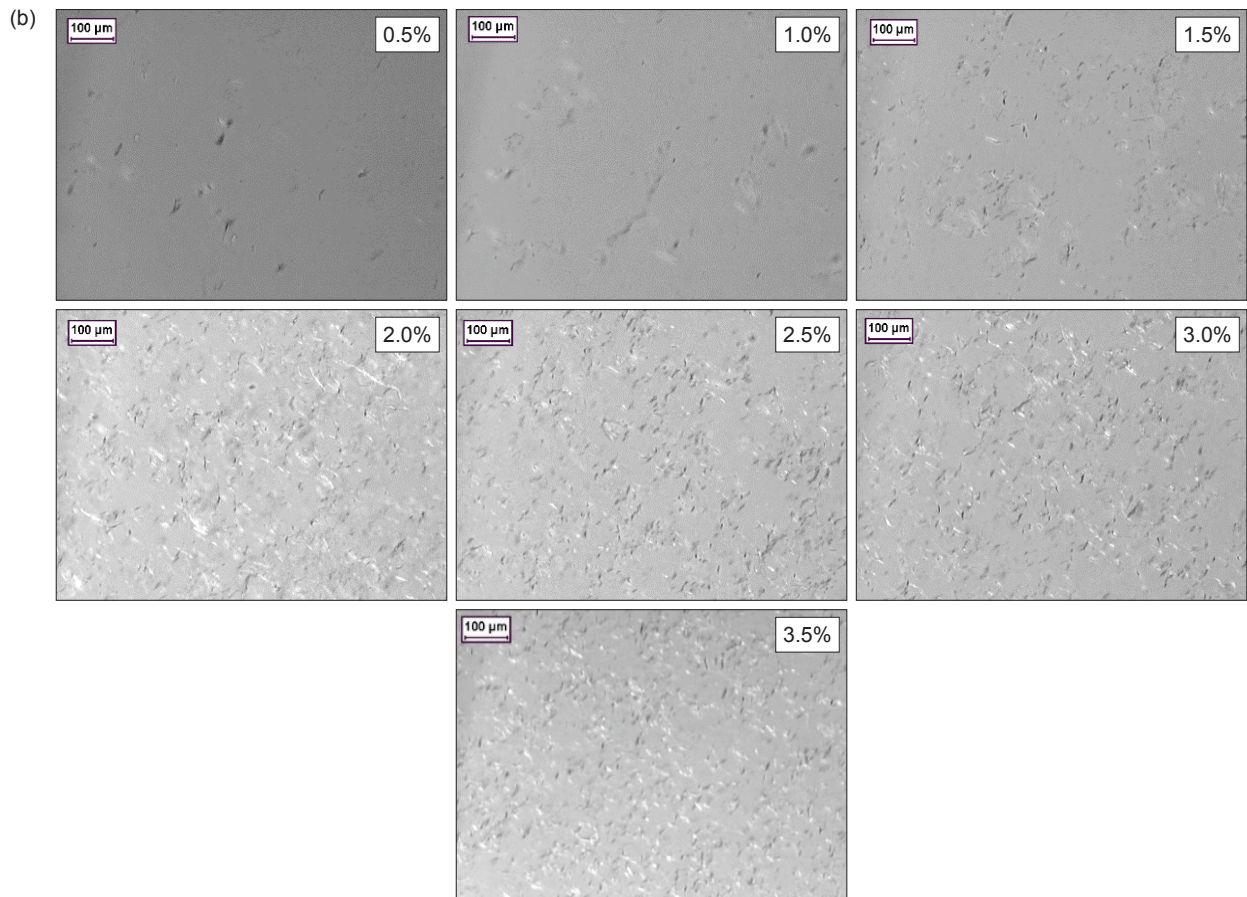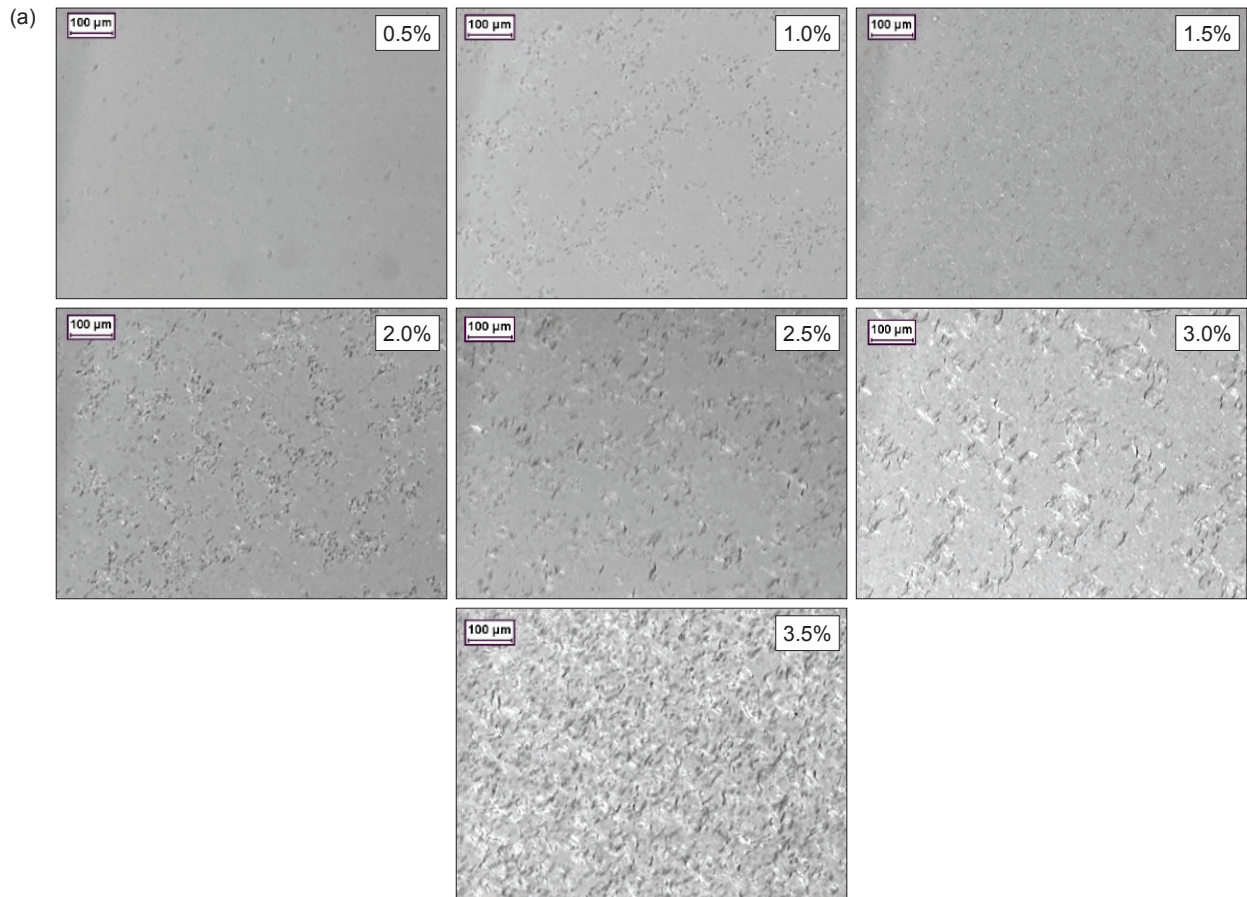
(a)

(b)

Figure 2. Polarised light micrographs (10x lens) of oil system with 0.5%-3.5% carnauba wax at (a) 10℃, (b) 25℃, (c) 40℃ and (d) 45℃.
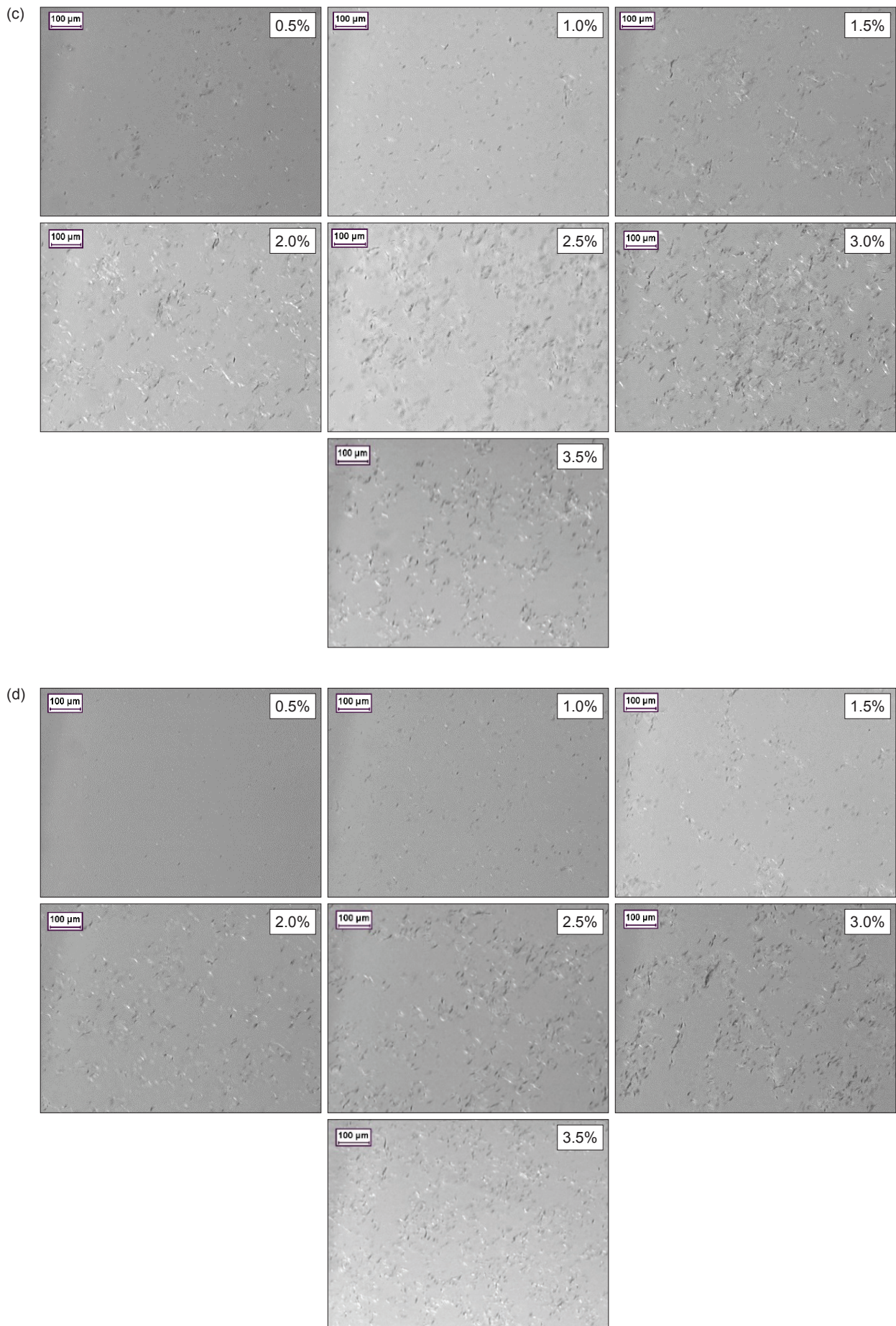
(a)

(b)

Figure 3. Polarised light micrographs (10x lens) of oil system with 0.5%-3.5% beeswax at (a) 10℃, (b) 25℃, (c) 40℃ and (d) 45℃.

The Effect of Waxes on Shear Viscosity of OSW

Shear viscosity of OSW with SFW, CW and BW at 0.5%-3.5% for 10°C, 25°C, 40°C and 45°C is shown in *Figure 4, 5* and *6*. At 5°C, shear viscosity of OSW with SFW was increased with increasing percentage of wax and all were statistically different ($p<0.05$). This OSW with SFW showed that non-Newtonian and shear thinning behaviour for all oil percentages as shear viscosity was decreased with increasing shear rate as wax thickened the oil system, except for 0.0% SFW. It was expected to observe that OSW with 3.0% SFW and 3.5% SFW produced the highest shear viscosity while the OSW with 0.5% SFW produced the lowest shear viscosity at 5°C at all experimental shear rates. Nevertheless, all shear viscosity of OSW with SFW was below 2.00 Pa.s. The shear viscosity was considered very low, and thus, should not affect spreadability. One of the commercial vegetable fats for confectionery spread was found to be 1.97 Pa.s for the shear rate at 100/s and about 6.00 Pa.s for the shear rate at 500/s at 30°C (Hadnađev *et al.*, 2011). According to Mazzanti *et al.* (2003), a low shear rate during cooling promoted the alignment of the gelator (10°C prior to the crystallisation onset temperature) during nucleation. However, the nuclei alignment and nucleation rate might change due to the high shear rate, which resulted in alteration of crystal in terms of size, distribution and population. This will consequently alter the strength of the gel (Blake and Marangoni, 2015; Da Pieve *et al.*, 2010; Doan *et al.*, 2018). At 10°C and 25°C, similar non-Newtonian and shear thinning behaviour were observed for OSW with 1.0%-3.5% SFW. However, the shear viscosity values were smaller than that observed at 5°C, especially at 100/s of shear rate with values of less than 1.30 Pa.s and 0.80 Pa.s, respectively. During shearing, heat and mass transfer occurred due to molecular collision which shattered the interaction between gelator, and thus damaged the linkage of crystals and altered the crystal size (Chopin-Doroteo *et al.*, 2011). A higher shear rate produced more heat and higher mass transfer occurred; hence, there was occurrence of shear thinning. Shear viscosity of OSW with 0.5% SFW at 10°C and 25°C were observed to have Newtonian behaviour. At 40°C and 45°C, the shear viscosity values were small, especially at the shear rates of 300/s and 400/s. At shear rate of 100/s, the shear viscosity for 40°C and 45°C were 0.48 Pa.s and 0.39 Pa.s, respectively for OSW with 3.5% SFW. OSW with 0.5% and 1.0% SFW were observed to have Newtonian behaviour while other systems showed non-Newtonian behaviour.
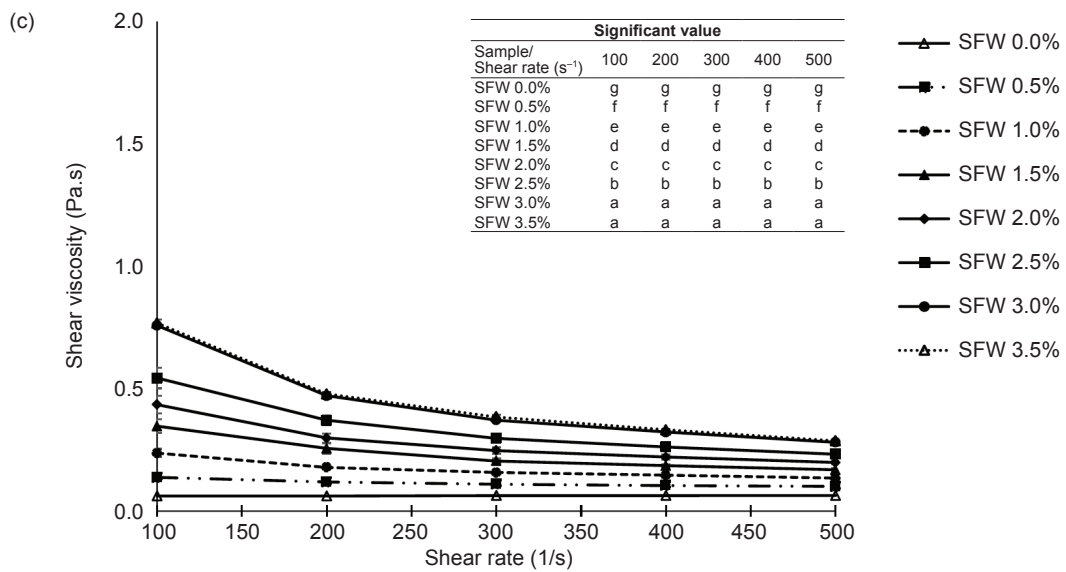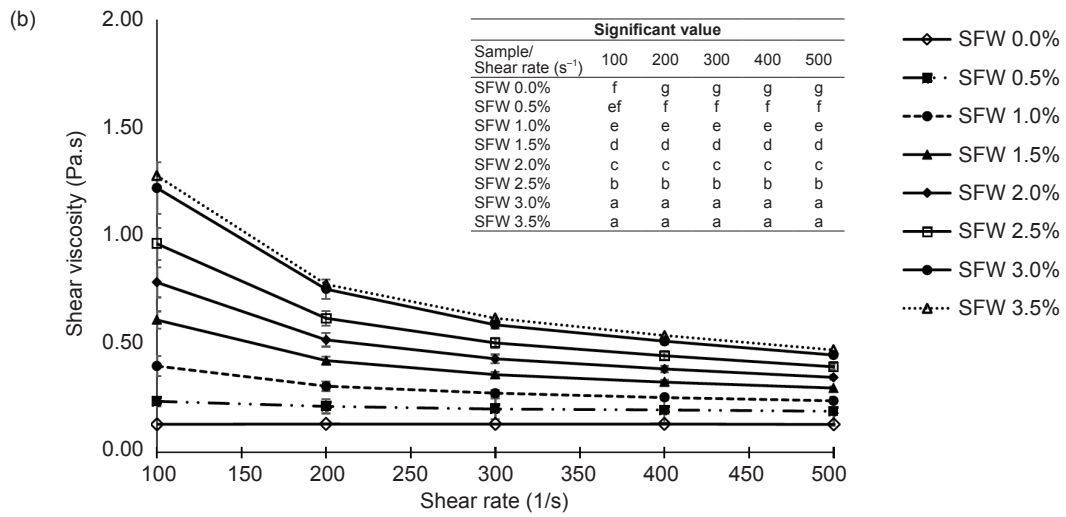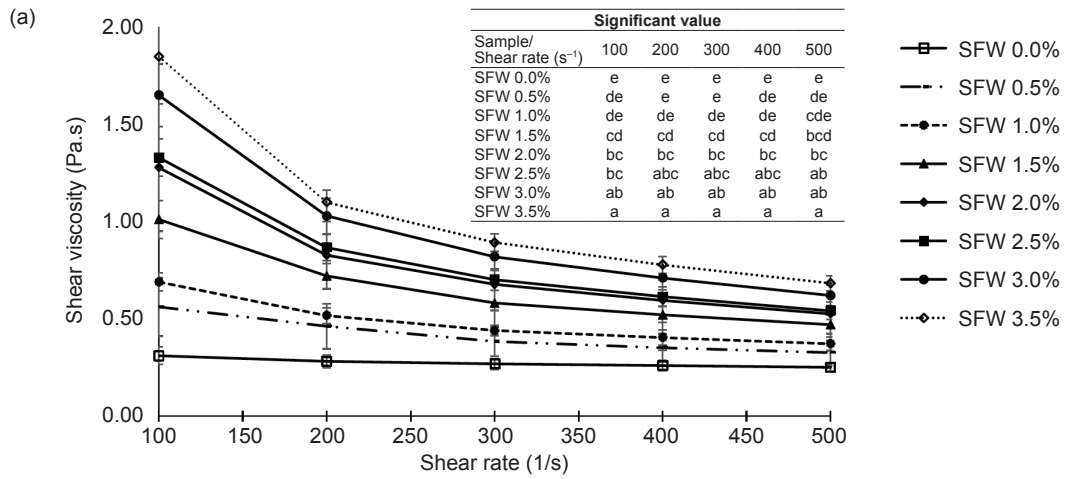
Shear viscosity values of OSW with CW at 5°C were small although they were significantly different ($p<0.05$). OSW with 0.5%-1.5% CW at 5°C showed Newto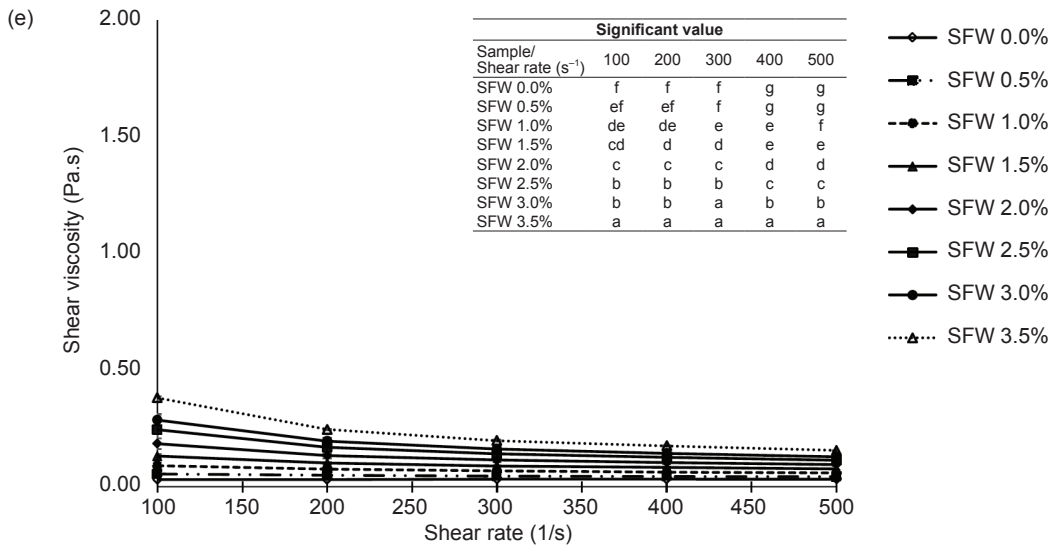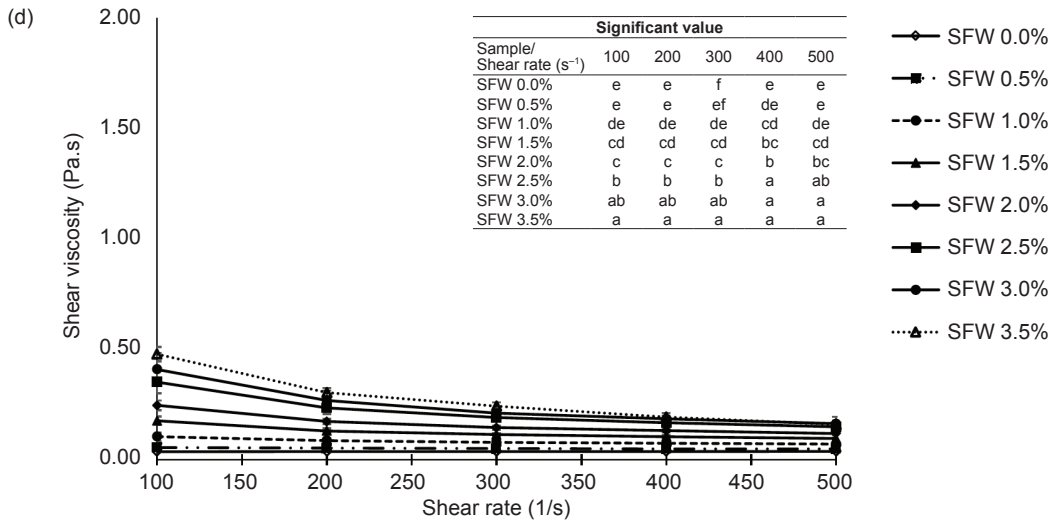nian behaviour while others showed non-Newtonian and shear thinning behaviour. Shear viscosity values of OSW with 3.5% CW at 100/s shear rate were 0.60 Pa.s and was well below the shear viscosity of OSW with 3.5% SFW. Therefore, spreadability at low temperature was possible. At 10°C, OSW with CW was comparable to 5°C. However, only OSW with 0.5% and 1.0% CW were observed to be Newtonian. At 25°C, 40°C and 45°C, the shear viscosity values were quite alike, with small shear viscosity for all percentages with less than 0.10 Pa.s.

At 5°C, shear viscosity of OSW with all percentages of BW were low although they were significantly different ($p<0.05$). Newtonian behaviour was observed for shear rate of 200/s - 500/s at 5°C. OSW with 3.5% BW was observed to have shear viscosity of less than 0.50 Pa.s for shear rate of 100/s; hence, will be the easiest to spread at low temperature for a spreadable product as compared to 3.5% SFW and 3.5% CW. At 10°C, 0.5% BW was observed to have Newtonian behaviour while other percentages showed non-Newtonian behaviour. Shear viscosity of OSW at 25°C, 40°C and 45°C were comparable for all percentages, except for 0.5%-1.5% BW with values of below 0.20 Pa.s. 0.5%-1.5% BW had shear viscosity of near 0 Pa.s for all shear rates.

CONCLUSION

FAC for all OSW was significantly different ($p<0.05$) although the FAC values were not much different from one another, indicating no major impact on saturation level for wax addition in base oil. In view of this, selection of any percentage of OSW might not affect the saturation level of the end product. Notwithstanding this, further selection of OSW was based on crystal structure and shear viscosity. Crystal structure of 3.5% waxes at 10°C showed that OSW with SFW was denser than CW followed by BW. OSW with 3.5% SFW might have difficulty in spreadability at low temperature (10°C). At 40°C and 45°C, crystal structures of OSW with 3.5% SFW were denser as compared to OSW of 3.5% CW and BW. Therefore, OSW with 3.5% SFW might potentially hinder the phase separation. In terms of shear viscosity at 5°C, OSW with 3.5% SFW had the highest value, followed by OSW with 3.5% CW and 3.5% BW. This demonstrated that OSW with 3.5% SFW was difficult to spread, which aligned with the finding of the crystal structure at low temperatures. At 40°C and 45°C, OSW with 3.5% SFW exhibited a higher shear viscosity value as compared to OSW with 3.5% CW and 3.5% BW. To choose a suitable OSW for the end product which requires spreadability at low temperature (5°C-10°C) and to minimise oil separation at high temperature (40°C-45°C), OSW with 3.5% CW might be the optimal choice.

(a)

(b)

(c)

(d)

Significant value					
Sample/ Shear rate (s⁻¹)	100	200	300	400	500
SFW 0.0%	e	e	f	e	e
SFW 0.5%	e	e	ef	de	e
SFW 1.0%	de	de	de	cd	de
SFW 1.5%	cd	cd	cd	bc	cd
SFW 2.0%	c	c	c	b	bc
SFW 2.5%	b	b	b	a	ab
SFW 3.0%	ab	ab	ab	a	a
SFW 3.5%	a	a	a	a	a

(e)

Significant value					
Sample/ Shear rate (s⁻¹)	100	200	300	400	500
SFW 0.0%	f	f	f	g	g
SFW 0.5%	ef	ef	f	g	g
SFW 1.0%	de	de	e	e	f
SFW 1.5%	cd	d	d	e	e
SFW 2.0%	c	c	c	d	d
SFW 2.5%	b	b	b	c	c
SFW 3.0%	b	b	a	b	b
SFW 3.5%	a	a	a	a	a

Note: Different small letters within each column in the table are significantly different ($p<0.05$).

Figure 4. Shear viscosity at different shear rates of oil system with various percentages of sunflower wax (SFW) at (a) 5°C (b) 10°C (c) 25°C (d) 40°C and (e) 45°C.

(a)

Significant value					
Sample/ Shear rate (s⁻¹)	100	200	300	400	500
CW 0.0%	d	d	d	de	de
CW 0.5%	d	d	d	e	e
CW 1.0%	cd	cd	cd	cde	cde
CW 1.5%	cd	cd	cd	bcde	bcde
CW 2.0%	bcd	bcd	bcd	bcd	bcd
CW 2.5%	bc	bc	abc	abc	abc
CW 3.0%	ab	ab	ab	ab	ab
CW 3.5%	a	a	a	a	a

(b)

	Significant value				
Sample/ Shear rate (s⁻¹)	100	200	300	400	500
CW 0.0%	d	c	d	d	d
CW 0.5%	cd	cd	cd	cd	cd
CW 1.0%	c	c	c	c	c
CW 1.5%	b	b	b	b	b
CW 2.0%	b	b	b	b	b
CW 2.5%	b	b	b	b	b
CW 3.0%	a	a	a	a	a
CW 3.5%	a	a	a	a	a

(c)

	Significant value				
Sample/ Shear rate (s⁻¹)	100	200	300	400	500
CW 0.0%	e	e	g	g	g
CW 0.5%	e	e	fg	fg	f
CW 1.0%	e	e	f	f	f
CW 1.5%	d	d	e	e	e
CW 2.0%	c	c	d	d	d
CW 2.5%	c	c	c	c	c
CW 3.0%	b	b	b	b	b
CW 3.5%	a	a	a	a	a

(d)

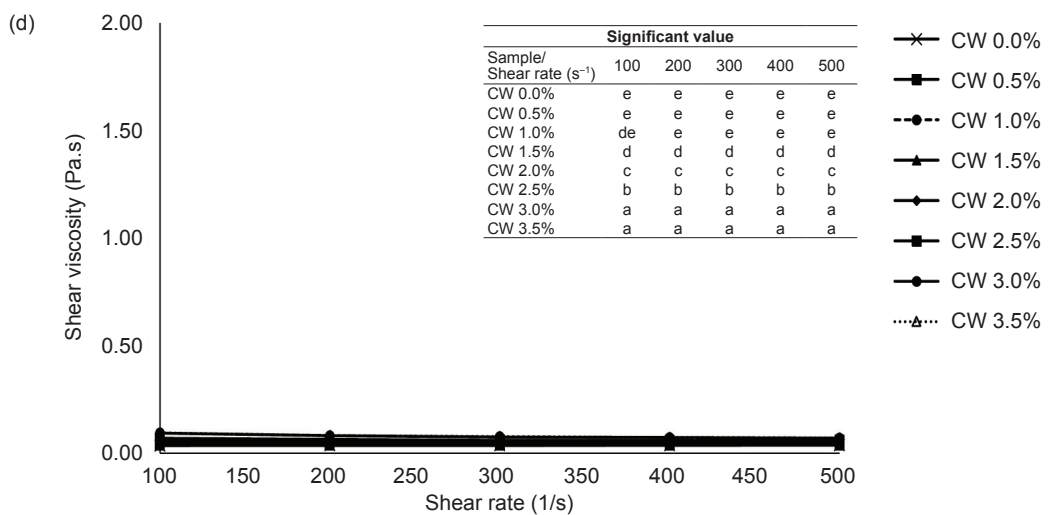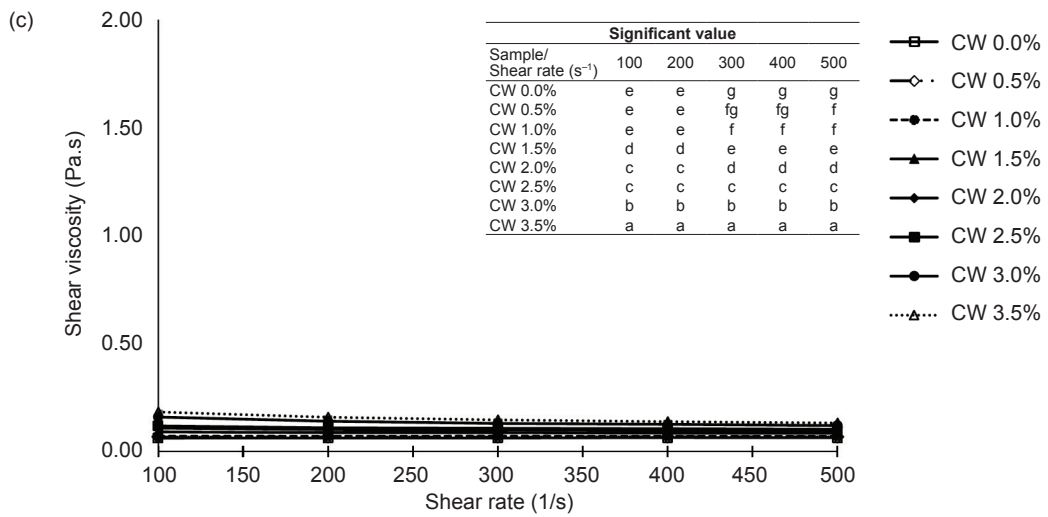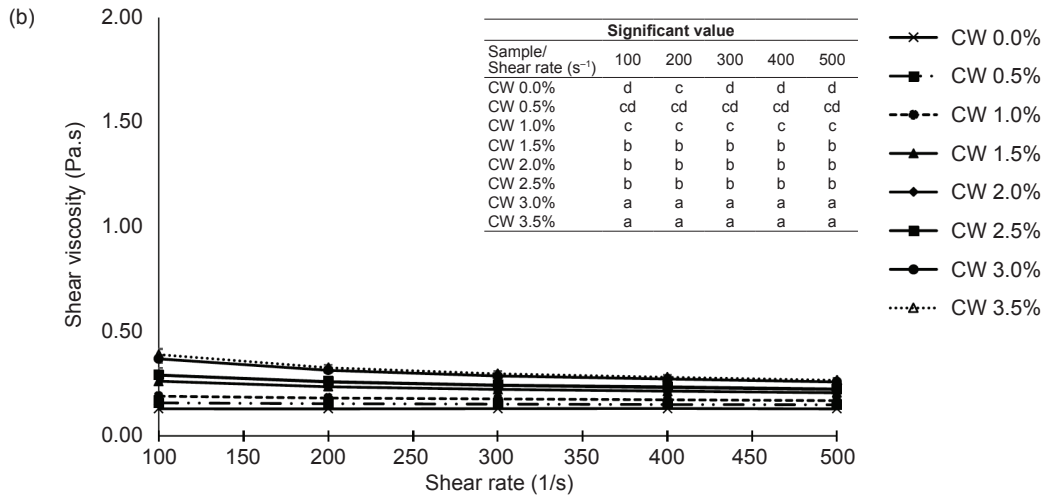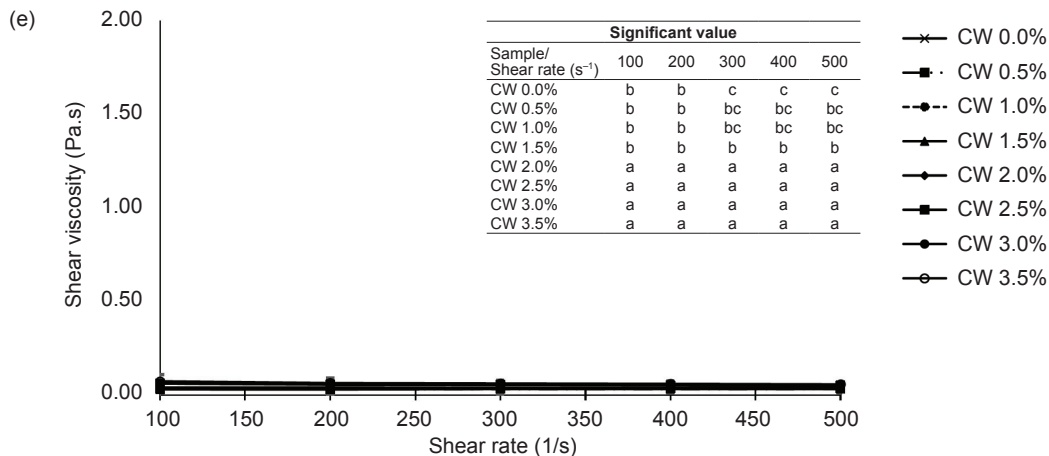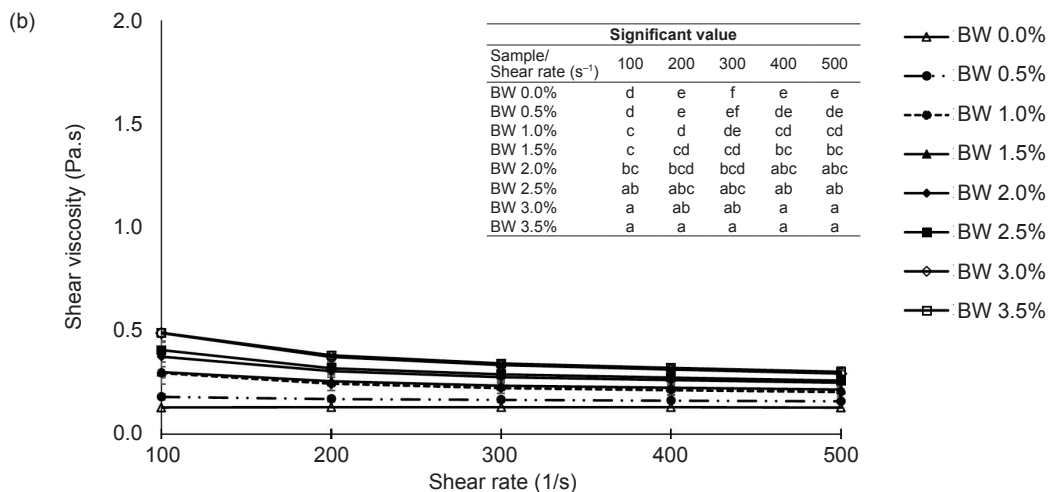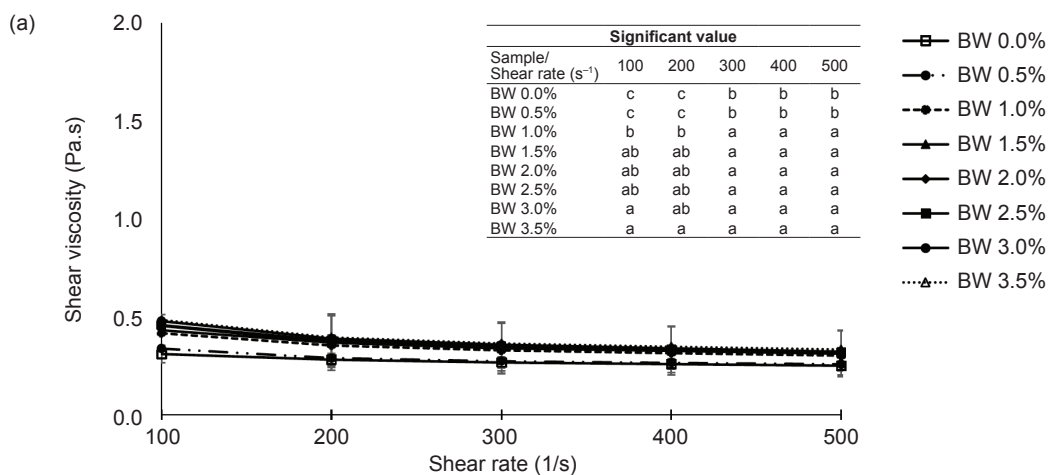|	**Significant value**				
Sample/ Shear rate (s⁻¹)	100	200	300	400	500
CW 0.0%	e	e	e	e	e
CW 0.5%	e	e	e	e	e
CW 1.0%	de	e	e	e	e
CW 1.5%	d	d	d	d	d
CW 2.0%	c	c	c	c	c
CW 2.5%	b	b	b	b	b
CW 3.0%	a	a	a	a	a
CW 3.5%	a	a	a	a	a

708

(e)

Note: Different small letters within each column in the table are significantly different ($p<0.05$).

Figure 5. Shear viscosity at different shear rate of oil system with various percentages of carnauba wax (CW) at (a) 5°C (b) 10°C (c) 25°C (d) 40°C and (e) 45°C.

(a)

(b)

(c)

Significant value					
Sample/ Shear rate (s⁻¹)	100	200	300	400	500
BW 0.0%	c	d	d	d	d
BW 0.5%	bc	d	d	d	d
BW 1.0%	bc	cd	cd	cd	cd
BW 1.5%	b	c	c	c	c
BW 2.0%	a	b	b	b	b
BW 2.5%	a	ab	ab	ab	ab
BW 3.0%	a	a	a	a	a
BW 3.5%	a	a	a	a	a

(d)

Significant value					
Sample/ Shear rate (s⁻¹)	100	200	300	400	500
BW 0.0%	e	e	e	e	f
BW 0.5%	de	de	de	de	ef
BW 1.0%	cd	d	d	d	e
BW 1.5%	c	c	c	c	d
BW 2.0%	b	b	b	b	c
BW 2.5%	b	b	b	b	c
BW 3.0%	a	a	a	a	b
BW 3.5%	a	a	a	a	a

(e)

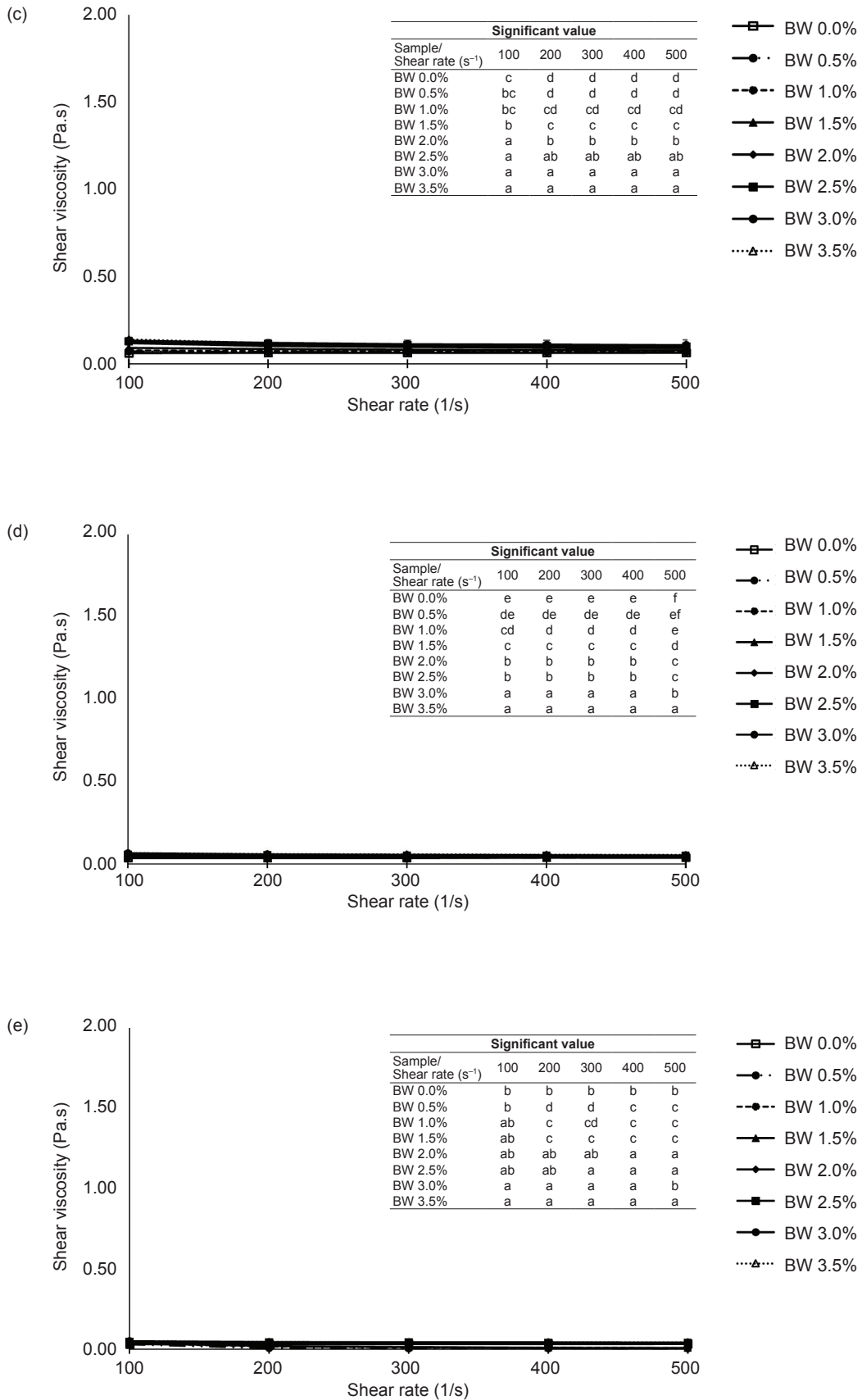| Significant value					
Sample/ Shear rate (s⁻¹)	100	200	300	400	500
BW 0.0%	b	b	b	b	b
BW 0.5%	b	d	d	c	c
BW 1.0%	ab	c	cd	c	c
BW 1.5%	ab	c	c	c	c
BW 2.0%	ab	ab	ab	a	a
BW 2.5%	ab	ab	a	a	a
BW 3.0%	a	a	a	a	b
BW 3.5%	a	a	a	a	a

Note: Different small letters within each column in the table are significantly different ($p<0.05$).

Figure 6. Shear viscosity at different shear rates of the oil system with various percentages of beeswax (BW) at (a) 5℃ (b) 10℃ (c) 25℃ (d) 40℃ and (e) 45℃.

ACKNOWLEDGEMENT

REFERENCES

Abdel-Razek, A G; Hassanein, M M M; Rudzinska, M and El-Mallah, M H (2017). Role of minor constituents and balanced fatty acids in upgrading the low stability of cooking oils blended with palm super olein. *Asian J. Sci. Res., 10*: 150-159.

Balasubramaniam, R; Damodar, G and Sughir, A (2012). Oleogel: A promising base for transdermal formulations. *Asian J. Pharm., 6:* 1-10.

Blake, A I and Marangoni, A G (2015). The effect of shear on the microstructure and oil binding capacity of wax crystal networks. *Food Biophys., 10*: 403-415.

Blake, A I; Co, E D and Marangoni, A G (2014). Structure and physical properties of plant wax crystal networks and their relationship to oil binding capacity. *JAOCS, 91*: 885-903.

Chai, X H; Meng, Z; Cao, P R; Liang, X Y; Piatko, M; Campbell, S; Koon Lo, S and Liu, Y F (2018). Influence of indigenous minor components on fat crystal network of fully hydrogenated palm kernel oil and fully hydrogenated coconut oil. *Food Chem., 255*: 49-57.

Chopin-Doroteo, M; Morales-Rueda, J A; Dibildox-Alvarado, E; Charó-Alonso, M A; de la Peña-Gil, A and Toro-Vazquez, J F (2011). The effect of shearing in the thermomechanical properties of candelilla wax and candelilla wax-tripalmitin organogels. *Food Biophys., 6*: 359-376.

Co, E D and Marangoni, A G (2012). Organogels: An alternative edible oil-structuring method. *JAOCS, 89*: 749-780.

Da Pieve, S; Calligaris, S; Nicoli, M C and Marangoni, A G (2010). Shear nanostructuring of monoglyceride organogels. *Food Biophys., 5*: 211-217.

Daniel, J and Rajasekharan, R (2003). Organogelation of plant oils and hydrocarbons by long-chain saturated FA, fatty alcohols, wax esters, and dicarboxylic acids. *JAOCS, 80*: 417-421.

Dassanayake, L S K; Kodali, D R; Ueno, S and Sato, K (2009). Physical properties of rice bran wax in bulk and organogels. *JAOCS, 86*: 1163-1173.

De Souza, R J; Mente, A; Maroleanu, A; Cozma, A I; Ha, V; Kishibe, T; Uleryk, E; Budylowski, P; Schünemann, H; Beyene, J and Anand, S S (2015). Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: Systematic review and meta-analysis of observational studies. *BMJ (Online), 351*: 1-16.

Doan, C D; Tavernier, I; Okuro, P K and Dewettinck, K (2018). Internal and external factors affecting the crystallization, gelation and applicability of wax-based oleogels in food industry. *Innov. Food Sci. Emerg. Technol., 45*: 42-52.

FDA (2018). Final determination regarding partially hydrogenated oils (removing *trans* fat). https://www.fda.gov/food/food-additives-petitions/final-determination regarding-partially-hydrogenated-oils-removing-trans-fat, accessed on 20 April 2020.

Gallego, R; Arteaga, J F; Valencia, C and Franco, J M (2013). Rheology and thermal degradation of isocyanate-functionalized methyl cellulose-based oleogels. *Carbohydr. Polym., 98*: 152-160.

Hadnađev, M; Hadnađev, T D; Torbica, A; Dokić, L; Pajin, B and Krstonošić, V (2011). Rheological properties of maltodextrin based fat-reduced confectionery spread systems. *Procedia Food Sci., 1*: 62-67.

Hughes, N E; Marangoni, A G; Wright, A J; Rogers, M A and Rush, J W E (2009). Potential food applications of edible oil organogels. *Trends Food Sci. Technol., 20*: 470-480.

Hwang, H S; Kim, S; Singh, M; Winkler-Moser, J K and Liu, S X (2012). Organogel formation of soybean oil with waxes. *JAOCS, 89*: 639-647.

Jang, A; Bae, W; Hwang, H-S; Lee, H G and Lee, S (2015). Evaluation of canola oil oleogels with candelilla wax as an alternative to shortening in baked goods. *Food Chem., 187*: 525-529.

Kim, J Y; Lim, J; Lee, J H; Hwang, H S and Lee, S (2017). Utilization of oleogels as a replacement for solid fat in aerated baked goods: Physicochemical, rheological, and tomographic characterization. *J. Food Sci., 8*: 445-452.

Kumar, R and Katare, O P (2005). Lecithin organogels as a potential phospholipid-structured system for topical drug delivery: A review. *AAPS J., 6*: 298-310.

Martins, A J; Cerqueira, M A; Cunha, R L and Vicente, A A (2017). Fortified beeswax oleogels:

Effect of *β*-carotene on the gel structure and oxidative stability. *Food Funct., 8*: 4241-4250.

Martins, A J; Cerqueira, M A; Fasolin, L H; Cunha, R L and Vicente, A A (2016). Beeswax organogels: Influence of gelator concentration and oil type in the gelation process. *Food Res. Int., 84*: 170-179.

Mazzanti, G; Guthrie, S E; Sirota, E B; Marangoni, A G and Idziak, S H (2003). Orientation and phase transitions of fat crystals under shear. *Crys. Growth Des., 3*: 721-725.

Moghtadaei, M; Soltanizadeh, N and Goli, S A H (2018). Production of sesame oil oleogels based on beeswax and application as partial substitutes of animal fat in beef burger. *Food Res. Int., 108*: 368-377.

MPOB (2005). Methods of test for palm oil and palm oil products. *MPOB Test Method.* MPOB, Bangi.

Ng, S P; Lai, O M; Abas, F; Lim, H K; Beh, B K; Ling, T C and Tan, C P (2014). Compositional and thermal characteristics of palm olein-based diacylglycerol in blends with palm super olein. *Food Res. Int., 55*: 62-69.

Norazura Aila, M H; Sivaruby, K; Nur Haqim, I; Noor Lida, H M D; Wan Rosnani, A I and Noor Soffalina, S S (2021). Palm-based chocolate spread for wide range temperature applications using sunflower wax, carnauba wax and bees wax. *J. Oil Palm Res.* DOI: 10.21894/jopr.2021.0051.

Öğütcü, M and Yilmaz, E (2014). Oleogels of virgin olive oil with carnauba wax and monoglyceride as spreadable products. *Grasas y Aceites, 65*: e040.

Öğütcü, M and Yılmaz, E (2015). Characterization of hazelnut oil oleogels prepared with sunflower and carnauba waxes. *Int. J. Food Prop., 18*: 1741-1755.

Patel, A R; Rajarethinem, P S; Grędowska, A; Turhan, O; Lesaffer, A; De Vos, W H; Van de Walle, D and Dewettinck, K (2014). Edible applications of shellac oleogels: Spreads, chocolate paste and cakes. *Food Funct., 5*: 645-652.

Patel, A R; Schatteman, D; De Vos, W H; Lesaffer, A and Dewettinck, K (2013). Preparation and rheological characterization of shellac oleogels and oleogel-based emulsions. *J. Colloid Interface Sci., 411*: 114-121.

Pehlivanoğlu, H; Demirci, M; Toker, O S; Konar, N; Karasu, S and Sagdic, O (2018). Oleogels, a promising structured oil for decreasing saturated fatty acid concentrations: Production and food-based applications. *Crit. Rev. Food Sci. Nutr., 58*: 1330-1341.

Romano, R; Giordano, A; Vitiello, S; Grottaglie, L L and Musso, S S (2012). Comparison of the frying performance of olive oil and palm superolein. *J. Food Sci., 77*: 519-531.

Rogers, M A; Strober, T; Bot, A; Toro Vazquez, J F; Stortz, T and Marangoni, A G (2014). Edible oleogels in molecular gastronomy. *Int. J. Gastron. Food Sci., 2*: 22-31.

Sánchez, R; Stringari, G B; Franco, J M; Valencia, C and Gallegos, C (2011). Use of chitin, chitosan and acylated derivatives as thickener agents of vegetable oils for bio-lubricant applications. *Carbohydr. Polym., 85*: 705-714.

Sandoval, J M; Carelli, A; Palla, C and Baümler, E (2020). Preparation and characterization of oleogel emulsions: A comparative study between the use of recovered and commercial sunflower waxes as structuring agent. *J. Food Sci., 85*: 2866-2878.

Singh, A; Auzanneau, F I and Rogers, M A (2017). Advances in edible oleogel technologies - A decade in review. *Food Res. Int., 97*: 307-317.

Stortz, T A; Zetzl, A K; Barbut, S; Cattaruzza, A and Marangoni, A G (2012). Edible oleogels in food products to help maximize health benefits and improve nutritional profiles. *Lipid Technol., 24*: 151-154.

Vintiloiu, A and Leroux, J C (2008). Organogels and their use in drug delivery - A review. *J. Control. Release, 125*: 179-192.

Wassell, P; Bonwick, G; Smith, C J; Almiron-Roig, E and Young, N W G (2010). Towards a multidisciplinary approach to structuring in reduced saturated fat-based systems - A review. *Int. J Food Sci., 45*: 642-655.

Zetzl, A K and Marangoni, A G (2014). Structured emulsions and edible oleogels as solutions to trans fat. *Trans Fats Replacement Solutions*. AOCS Press. p. 215-243.