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Physicochemical properties of soybean-based diacylglycerol before and after dry fractionation

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Abstract

In the present work, the physicochemical properties of soybean-based DAG (SDAG), especially the effects of dry fractionation on crystallisation properties, were investigated. The solid portion (SF) and liquid portion (LF) were obtained by dry fractionation of SDAG. The physicochemical properties of soybean oil, SDAG, LF, and SF were investigated, including the determination of acylglycerol composition, fatty acid (FA) composition, slip melting point (SMP), iodine value (IV), solid fat content (SFC), X-ray diffraction (XRD), and differential calorimetry (DSC). The DAG content of SF (44.79 ± 0.87%) was higher than SDAG (41.94 ± 1.28%) and LF (42.22 ± 0.29%). After fractionation, SF contained higher levels ($p < 0.05$) of saturated fatty acids (SAFA) and lower levels ($p < 0.05$) of unsaturated fatty acids (UFA) than LF and SDAG, which was in accordance with the differences in IV and SMP. The SFC of SF (0°C, 17.25 ± 0.65%; 20°C, 10.66 ± 0.28%; 40°C, 3.47 ± 0.14%) was significantly higher than those of LF and SDAG ($p < 0.05$). With respect to the crystal structures, it was demonstrated that SF contained more β' crystals than SDAG and LF and could be better used in shortening. Overall, the present work provides a theoretical basis for the industrial applications of SDAG and its fractions.

Introduction

In recent decades, fats and oils are highly consumed which results in an increased obesity in the population and leads to a series of chronic diseases (Kim *et al.,* 2017). Diacylglycerol (DAG) has significant effects in preventing body fat accumulation (Latip *et al.,* 2013a) and lowering postprandial lipids (Zhang *et al.,* 2019). The beneficial effects of DAG are related to a different metabolic pathway as compared to that associated with triacylglycerol, (TAG) (Saberi *et al.,* 2011). Thus, DAG has great potential in treating or preventing chronic diseases associated with obesity. In addition, it has been reported that DAG has some other benefits such as improving bone health (Lee *et al.,* 2019), decreasing insulin resistance in type II diabetic patients (Zheng *et al.,* 2015), and improving the β-oxidation of fatty acids (FAs) (Ota *et al.,* 2007). DAG oil is very similar to traditional edible oils (TAG) in taste and appearance. Therefore, DAG serves as a promising alternative to the traditional TAG (Li *et al.,* 2015). However, common plant and animal oils contain less than 10% (w/w) DAG (Saito *et al.,* 2017). Today, DAG is generally produced chemically or enzymatically.

DAG can be produced from many different sources of plant oil or animal fats, including palm oil, lard, milk fat, canola oil, and soybean oil (Xu *et al.,* 2016; Zhao *et al.,* 2020). DAG prepared from soybean oil has good nutritional value because it provides high level of unsaturated and essential FAs (Chen *et al.,* 2020). Unexpectedly, carcinogenic glycidyl esters (GEs) were identified in DAG products in 2009, leading to strictly banned commercial availability thereafter (Haines *et al.,* 2011).

The physicochemical properties of DAG are different from those of TAG due to a free hydroxyl group on the glycerol backbone (Saberi *et al.,* 2011). As compared to TAG, DAG samples with the same FA compositions have higher melting points and different crystallisation properties (Xu *et al.,* 2016). The taste of such sample is closely associated with these physicochemical parameters. Dry fractionation is a simple and effective way to improve the quality of oils and fats. Additionally, physicochemical properties can also be

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changed during dry fractionation (Bootello *et al.,* 2011). Dry fractionation of SDAG can separate the solid portion (SF) and liquid portion (LF) of SDAG.

In previous studies, the physicochemical properties of palm oil-based DAG, rapeseed oil-based DAG, and lard-based DAG have been comprehensively studied (Zaliha *et al.,* 2004; Saberi *et al.,* 2011; Ng *et al.,* 2014). To the best of our knowledge, research on the physicochemical properties of SDAG is still limited, especially regarding the differences of fractions. In the present work, the physicochemical properties of soybean oil, SDAG, and its fractions were investigated, including the acylglycerol profile, FA composition, slip melting point (SMP), crystallisation properties, and content of GE and 3-monochloropropane-1,2-diol esters (3-MCPDE). It is believed that the present work will contribute to the industrial applications of SDAG and its fractions.

Materials and methods

Materials and chemicals

Soybean oil was purchased from Yihai Kerry Golden Arowana Co. Ltd. (Guangdong, China). SDAG was supplied by Guangdong Yue-shan Special Nutrition Technology Co. Ltd. (Guangdong, China). Glycidyl palmitate, glycidyl stearate, glycidyl oleate, glycidyl linoleate, 3-MCPDE, 1,3-dipalmitoyl-3-chloropropanediol-d_5 (PP-3-MCPD-d_5), 1,3-dipalmitoyl-3-chloropropanediol (PP-3-MCPD), triolein, diolein, monoolein, and fatty acid methyl ester standards were purchased from Aladdin (Shanghai, China). All other reagents, such as isopropanol, isooctane, formic acid, and *n*-hexane were of HPLC-grade and purchased from Aladdin (Shanghai, China).

Dry fractionation of SDAG

Dry fractionation is used for the physical modification of oil (Koay *et al.,* 2013). The DAG fractionation process was based on the previous study (not published). SDAG was melted at 50°C, and transferred to a thermostat water bath (GongYi City YuHua Instrument Co. Ltd., Zhengzhou, China). Dry fractionation was performed with an initial temperature of 25.4°C and the system was cooled slowly and uniformly to 8.9°C within 6.5 h. Subsequently, the same temperature was maintained for 3 h. The SF and LF were obtained after the filtering process using a Brinell funnel. The samples were stored at 4°C before analysis. The fractionation process was repeated three times.

Acylglycerol profile

The acylglycerol composition of soybean oil, SDAG, LF, and SF were analysed following the method described by Li *et al.* (2019) with slight modifications. The determination was carried on with HPLC (Waters Corporation, Milford, MA, USA) and a refractive index detector (Waters Corporation, Milford, MA, USA). Briefly, 1 mL mobile phase (*n*-hexane, 2-propanol, and formic acid, 18:1:0.003, v/v/v), 30 µL sample, and a certain content of anhydrous sodium sulphate were added to a 2 mL vial. Then, the supernatant was collected and used for HPLC injection following centrifugation (10,000 *g*, 3 min). The sample was separated by a Phenomenex Luna column (250 × 4.6 mm i.d., 5 µm particle size, Phenomenex Corporation, Torrance, CA, USA) at 35°C. The flow rate of the mobile phase was 1 mL/min. The peaks in the HPLC profile were analysed based on the retention times of known standards. The proportions of TAG, DAG, MAG, and FFA were obtained based on the area normalisation method.

Iodine value

The analysis of the iodine value was carried out following Cd 1d-92 method of AOCS (1997a).

Slip melting point

The analysis of the slip melting point was carried out following method Cc 3-25 of AOCS (1997b).

Fatty acid composition

The FA composition of the soybean oil, SDAG, LF, and SF were analysed by gas chromatography (GC) coupled with a flame ionisation detector (FID) (Agilent 7890A, Agilent Technologies, CA, USA). The analysis was carried out following Ce 2-66 method of AOCS (1997c) with slight modifications. Briefly, 300 µL sample and 6 mL methanolic sodium hydroxide solution (2%) were added to a 50 mL flask, and heated at 60°C for 30 min. Then, 3 mL methanolic boron trifluoride solution was added, and the flask was kept at 60°C for 5 min. The mixed solution was cooled to room temperature, and then 5 mL isooctane and 10 mL saturated sodium chloride solution were added. Anhydrous sodium sulphate was added to the upper layer to remove the water. The final obtained solution was used for GC injection following filtration with 0.45 µm filter membrane. The FA composition was obtained based on the area normalisation method.

GE and 3-MCPDE content

The GE (glycidyl palmitate, glycidyl stearate, glycidyl oleate, and glycidyl linoleate) contents of SDAG, LF, and SF were analysed by liquid chromatography and mass spectrometry (RRLC-QQQ, Agilent Technologies, CA, USA) (Blumhorst *et al.,* 2011). The

3-MCPDE contents of SDAG, LF, and SF were analysed according to Li *et al.* (2019), and were determined by an Agilent 7890A GC fitted with a 5975C mass selective detector (Agilent 7890A, Agilent Technologies, CA, USA).

Solid fat content

The SFC was determined using a pulsed nuclear magnetic resonance (p-NMR) spectrometer (Minispec-mq20, Bruker, Karlsruhe, Germany) according to Podchong *et al.* (2018) with slight modifications. Briefly, each sample (3 mL) was poured into an NMR tube and incubated at 80°C for 40 min to eliminate the formed crystallisation. Then, the samples were placed at 0°C for 90 min to crystallise totally before measurement. The SFC was determined in the range of 0 - 40°C (5°C interval). All samples were kept at the measurement temperature for 30 min before the SFC was determined.

Polymorphism analysis

The polymorphisms of the SDAG, LF, and SF were determined by an X-ray diffractometer (Xpert powder 3, Panalytical, Almelo, Netherlands) according to Podchong *et al.* (2018). Briefly, samples were incubated at 80°C for 40 min to eliminate historical crystallisation, cooled down to -18°C, and kept for 1 h for total crystallisation. Samples were placed in the sample holder for analysis, and the data were analysed with the software "Xpert Data Collector".

Differential scanning calorimetry analysis

The crystal melting and crystallisation curves of SDAG, LF, and SF were analysed using a DSC (HS-DSC-101, HESON, Shanghai, China). The instrument was calibrated with indium (Duan *et al.,* 2020). From each sample, approximately 6 - 10 mg was sealed into an aluminium pan, with an empty sealed pan as a blank. The temperature was programmed according to Tavernier *et al.* (2019) with slight modifications. Each sample was heated to 50°C, and kept for 10 min to eliminate the formed crystallisation, and cooled to -40°C at a speed of 5°C/min. After holding for 10 min at -40°C, the temperature was raised to 40°C at 5°C/min. During the process, nitrogen was used at a flow rate of 50 mL/min to prevent oxidation.

Statistical analysis

All experiments were repeated in triplicate, and all data were expressed as means ± standard deviations. The difference between measured values of samples was evaluated by one-way analysis of variance (ANOVA) with two-tailed Student's *t*-test ($p < 0.05$).

Results and discussion

Physicochemical characterisations

The acylglycerol profile, FA composition, SMP, and IV are shown in Table 1. The DAG contents of SDAG and LF were 41.94 ± 1.28 and 42.22 ± 0.29%, respectively, which were not significantly different ($p > 0.05$). SF had a higher DAG content (44.79 ± 0.87%) than SDAG and LF ($p < 0.05$). This difference might be associated with the higher documented melting point of DAG than TAG at the same FA compositions (Xu *et al.,* 2016).

The main FAs of SDAG and its fractions were oleic (~ 25%), linoleic (~ 51%), palmitic (~ 8%), and stearic (~ 4%) acids, which is similar to the FA composition of soybean oil (Table 1). As expected, a high content of UFA was observed even after the fractionation process (Chen *et al.,* 2020).

After dry fractionation, the content of UFA (oleic and linoleic acids) in SF significantly decreased, while the content of SAFA (palmitic and stearic acids) significantly increased as compared to the values in SDAG and LF ($p < 0.05$). DAG with more SAFA had higher melting points, and could be more easily separated into SF. However, there were no significant differences between the proportions of the main FAs in LF and SDAG ($p > 0.05$). The yield ratio of SF to LF was 4.72%:95.28%, and the proportion of SF was relatively low, thus, dry fractionation did not affect the FA composition in LF. The results are in accordance with the report of Podchong *et al.* (2018).

The IV value of SF (117.58 ± 2.39% I_2/100 g) was lower than those of LF (128.53 ± 2.26 I_2/100 g), SDAG (127.08 ± 2.61 I_2/100 g), and soybean oil (125.32 ± 2.43 I_2/100 g) ($p < 0.05$), which is in accordance with the results of a previous report (Zaliha *et al.,* 2004). This observation was indicative of a decreased level of UFA in SF, and was in accordance with the FA compositions described earlier.

The SMP results of SDAG, LF, and SF are exhibited in Table 1. The SMP of SF was 34 ± 0.1°C. As expected, this level was much higher than those of LF (0.5 ± 0.1°C) and SDAG (1.2 ± 0.1°C). This result might be associated with the higher SAFA content in SF.

3-MCPDE and GE content

3-MCPDEs and GEs are the main contaminants during DAG production (Cheng *et al.,* 2016; Yao *et al.,* 2019). The sales of DAG were prohibited due to contamination by GEs in 2009. Thus, the content of 3-MCPDE and GE are important indicator reflecting the safety of DAG oil. As shown in Table 2, 3-MCPDEs and GEs (mainly glycidyl palmitate,

Table 1. The physicochemical of soybean oil, SDAG, LF, and SF.

Composition	Soybean oil	SDAG	LF	SF
TAG (%)	98.69 ± 0.59[a]	58.00 ± 0.41[b]	57.59 ± 0.39[b]	54.96 ± 0.48[c]
DAG (%)	1.28 ± 0.02[c]	41.95 ± 1.28[b]	42.23 ± 0.29[b]	44.83 ± 0.87[a]
MAG (%)	N.D.	N.D.	N.D.	N.D.
FFA (%)	0.03 ± 0.001[b]	0.05 ± 0.001[b]	0.18 ± 0.003[a]	0.21 ± 0.002[a]
Iodine value (I_2/100 g)	125.32 ± 2.43[a]	127.08 ± 2.61[a]	128.53 ± 2.26[a]	117.58 ± 2.39[a]
Slip melting point (°C)	N.D.	1.2 ± 0.1[b]	0.5 ± 0.1[b]	34.0 ± 0.1[a]
C16:0	10.68 ± 1.09[a]	9.04 ± 0.15[b]	9.02 ± 0.05[b]	11.04 ± 0.41[a]
C18:0	4.50 ± 0.13[b]	4.35 ± 0.03[b]	4.31 ± 0.03[b]	5.82 ± 0.26[a]
C18:1n9	25.82 ± 0.35[a]	25.25 ± 0.22[a]	25.37 ± 0.18[a]	24.45 ± 0.17[b]
C18:2n6	52.09 ± 0.37[b]	54.21 ± 0.81[a]	54.18 ± 0.36[a]	51.85 ± 0.21[b]
γ-C18:3n6	0.17 ± 0.01[a]	0.17 ± 0.02[a]	0.17 ± 0.01[a]	0.16 ± 0.03[a]
α-C18:3n3	6.74 ± 0.03[a]	6.98 ± 0.13[a]	6.95 ± 0.15[a]	6.68 ± 0.27[a]

The significance analysis was mainly comparing the differences of the same indicators between the above four samples. Limit of detection: MAG - 0.05%. SDAG: soybean-based DAG; LF: liquid portion obtained from the dry fractionation of SDAG; SF: solid portion obtained from the dry fractionation of SDAG; TAG: triacylglycerol; DAG: diacylglycerol; MAG: monoglyceride; FFA: free fatty acid; C 16:0: palmitic; C 18:0: stearic acid; C18:1n9: oleic acid; C18:2n6: linoleic acid; γ-C18:3n6: β-linolenic acid; and α-C18:3n3: α-linolenic acid.

glycidyl stearate, glycidyl oleate, and glycidyl linoleate) were not detected in SDAG. The limit of detection and limit of quantification of 3-MCPDEs were 0.10 and 0.30 mg/kg, respectively. The limit of detection and limit of quantification of GEs are shown in Table 3.

Solid fat content

SFC is an important index affecting the application of DAG products (Saberi *et al.,* 2011). The SFC profiles of SDAG and fractions are shown in Figure 1. The SFC values of SF were higher than those of SDAG and LF ($p < 0.05$), indicating a higher SMP. At 0°C, the SFC of SF was 17.25 ± 0.65%. Increased temperature led to the decrease in SFC values. The SFC was 10.66 ± 0.28% at 20°C, and decreased to 3.47 ± 0.14% at 40°C. It has been documented that the oiling off in shortening is greatly affected by SFC at 20°C (Laia *et al.,* 2000; Wassell and Young, 2007; Podchong *et al.,* 2018). The data showed that only a small amount of solid fat was observed in SF at 37°C. Therefore, SF had good melting at body temperature (Podchong *et al.,* 2018). In addition, it has been reported that the SFC at body temperature (< 3.5%) was associated with smooth texture (Chrysan, 2005). Overall, it is anticipated that SF could be used in shortening.

Table 2. The content of GEs and 3-MCPDE of SDAG, LF, and SF.

Index	SDAG	LF	SF
GEs (μg/Kg)	N.D.	N.D.	N.D.
3-MCPD (μg/Kg)	N.D.	N.D.	N.D.

GEs: glycidyl esters; 3-MCPD: 3-monochloropropane-1,2-diol esters; SDAG: soybean-based DAG; LF: liquid portion obtained from the dry fractionation of SDAG; SF: solid portion obtained from the dry fractionation of SDAG; and N.D.: not detected.

Table 3. Limit of detection (LOD) and limit of quantification (LOQ) of GEs.

GEs	LOD	LOQ
glycidyl palmitate	0.013	0.028
glycidyl stearate	0.011	0.018
glycidyl oleate	0.026	0.032
glycidyl linoleate	0.010	0.021

GEs: glycidyl esters.

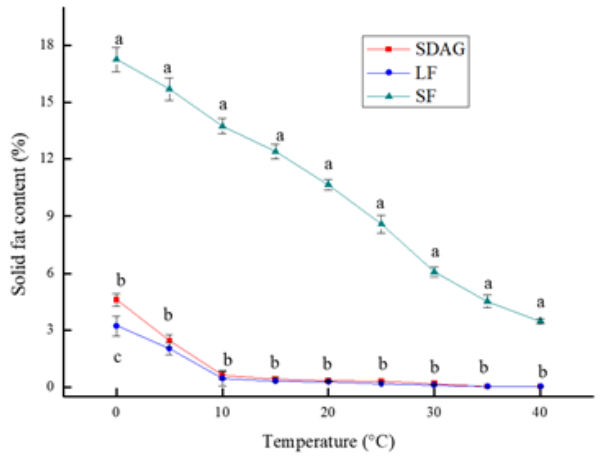

Figure 1. Solid fat content of SDAG, LF, and SF from 0 to 40°C. SDAG: soybean-based DAG; LF: liquid portion obtained from the dry fractionation of SDAG; and SF: solid portion obtained from the dry fractionation of SDAG.

SDAG and LF had a lower SFC and a higher SMP than SF. At 0°C, the SFC of LF was 3.22 ± 0.53%, lower than that of SDAG (4.59 ± 0.33%). The SFC values of LF and SDAG showed striking changes at temperatures between 0 and 10°C. At temperatures higher than 10°C, the SFC values of LF and SDAG decreased to 0%, thereby indicating that the SFCs of LF and SDAG were too low and not suitable for making shortening or margarine. However, materials with these SFCs could be used in emulsion products such as mayonnaise (Boode *et al.,* 1993). It is documented that low SFC values could contribute to improved emulsion stability (Boode *et al.,* 1993). The results correspond to a report regarding the dry fractionation of palm-based DAG (Latip *et al.,* 2013a).

X-ray diffraction analysis

It is generally acknowledged that the polymorphic forms of DAG oil are associated with its sensory attributes. In the present work, this key parameter was determined according to Yang *et al.* (2004) and Miklos *et al.* (2013). The polymorphic forms were analysed based on the following information: the 2θ value of the α form is approximately 21°, with a corresponding short spacing of 4.15 Å; the 2θ values of the β' form is approximately 20.8° and 23.0°, with corresponding short spacings of 4.2 and 3.8 Å, respectively; the 2θ value of the β-crystal form is approximately 19.1°, with a corresponding short spacing of 4.6 Å.

The polymorphic forms of the SDAG and SF at 25°C are shown in Figure 2. It was found that LF could not crystallise effectively at 25°C. There were no peaks at 20.8° and 23° on the SDAG spectrum, indicating that there were only β, and no β' forms, while SF consisted of β and β' forms. Podchong *et al.* (2018) reported that palm stearin and its fractions all existed as mixtures of β' and β crystals. The difference was that the melting point of palm oil was lower than that of SDAG and could crystallise at room temperature.

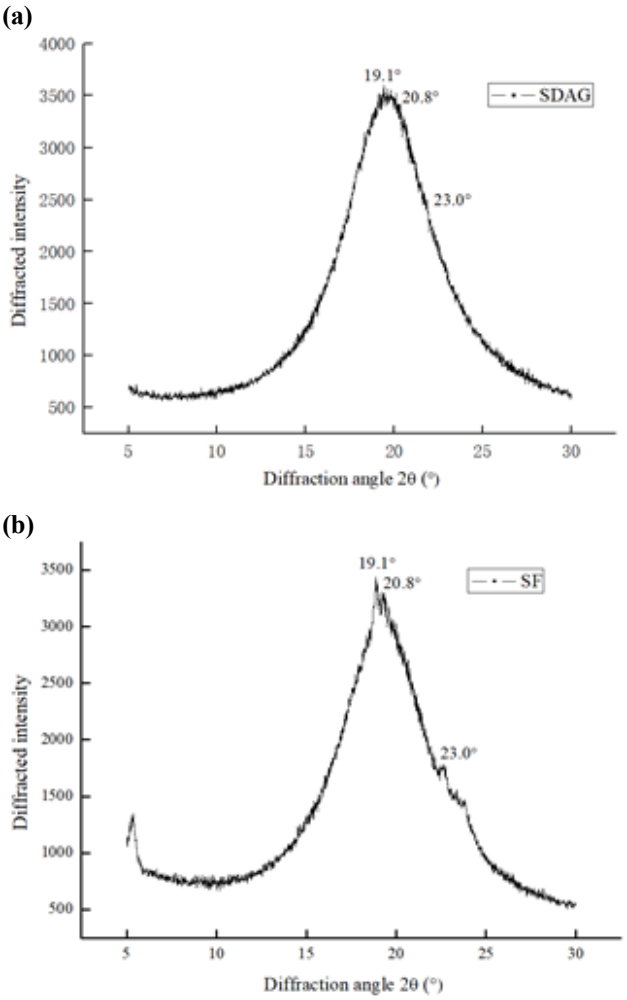

Figure 2. X-ray diffraction spectrum of (a) SDAG, and (b) SF. SDAG: soybean-based DAG; and SF: solid portion obtained from the dry fractionation of SDAG.

The α form is the most unstable polymorphic form and can easily transform into other polymorphic forms. The β and β' forms are relatively stable and β' forms are related to smooth texture (Svenstrup *et al.,* 2005). In contrast, more β forms are usually associated with a coarse, grainy, and dull texture (Lida and Ali, 1998). Therefore, β' has been generally regarded as the most ideal crystal form, especially for shortening. DAG oil with more β' forms could integrate more liquid portions into the crystal networks, and decrease the oil-exudation phenomenon (Saberi *et al.,* 2011). It has been reported that a higher structural stability of the β'-crystal form could be observed in

DAG with higher contents of palmitic acid (Chawla and Deman, 1994). In the present work, it was found that SF contained more palmitic acids (10.93 ± 0.41%) than did SDAG (8.97 ± 0.15%) and LF (8.95 ± 0.05%). These data suggest that SF is endowed with a smoother texture. Consequently, SF is suitable for shortening, while SDAG or LF is more suitable to be used as dough lubricant/spraying oil (Lida and Ali, 1998).

Differential scanning calorimetry

The DSC melting and crystallisation curves of SDAG, SF, and LF are shown in Figure 3. As shown in Figure 3(a), the crystallisation curves of soybean oil showed wide melting point range at approximately -17.9°C, which was attributed to the co-crystallisation of TAG composed of different FA compositions. SDAG had two crystallisation peaks at -3.3 and 8.5°C, which were related to the TAG and DAG components, respectively (Saberi *et al.,* 2011; Xu *et al.,* 2016). The crystallisation curves of LF did not show significant differences from those of SDAG ($p > 0.05$). Unexpectedly, the first peak of SF was observed at a lower temperature (-8.9°C). The second peak of SF was found at a higher temperature (11.0°C) which might be related to the higher melting point of SF.

As shown in Figure 3(b), the melting curves of soybean oil showed one major broad endotherm peak at -13.3°C, which was associated with different FA compositions of the TAGs in soybean oil, resulting in a wide melting point range (Zaliha *et al.,* 2004; Latip *et al.,* 2013b). SDAG exhibited two melting peaks. The first peak was at -22.0°C which was related to the TAG component; the second peak was at -9.6°C, which was associated with the DAG component. Although the DAG and TAG shared similar FA compositions, the former showed a higher melting point than the latter (Xu *et al.,* 2016). The melting curve of LF demonstrated that LF melted at -11.1°C and only had one melting peak. This observation could be explained by the fact that the components with high melting points were separated through dry fractionation. In contrast, three melting peaks could be found in DSC curve of SF. The first peak was at -30.9°C, which might be associated with the lower melting point components, including glycerol trioleate, glycerol trilinoleate, glycerol dioleate, and glycerol dilinoleate. The second peak was at -3.9°C, which might be related to TAG or DAG composed of mixed FAs. The third peak was at 31.5°C, which was much higher than the melting point of the LF. This phenomenon might be due to the high level of SAFA (*e.g.*, palmitic and stearic acids) in DAG.

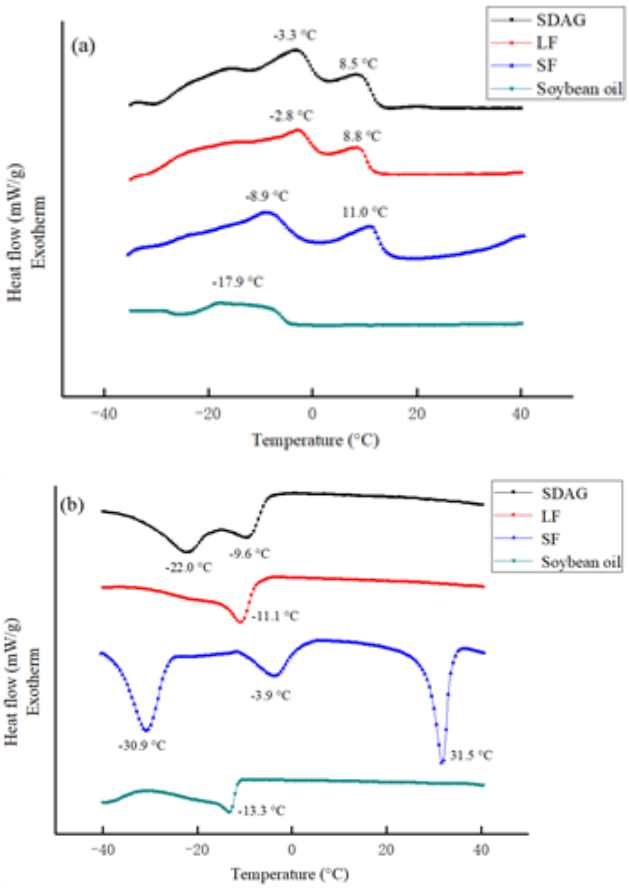

Figure 3. (a) Crystallisation curve, and (b) melting curve of soybean oil of SDAG, LF, and SF samples. SDAG: soybean-based DAG; LF: liquid portion obtained from the dry fractionation of SDAG; and SF: solid portion obtained from the dry fractionation of SDAG.

Conclusion

The physicochemical properties of SDAG and its fractions were significantly affected by dry fractionation. SF contained more SAFA (palmitic and stearic acids) and less UFA (oleic and linoleic acids) than did SDAG, which was in accordance with the IV results. SF had a higher SFC (0°C, 17.25 ± 0.65%; 20°C, 10.66 ± 0.28%; 40°C, 3.47 ± 0.14%) and contained more β'-crystals at 25°C than LF and SDAG, thus, SF was suitable in producing shortening or margarine. SDAG and LF had a lower SFC (0°C, 3.22 ± 0.53%; 10°C, 0%) and contained only β crystals, thus, they could be used in emulsion products (*e.g.*, mayonnaise) or as dough lubricant/spraying oil. The insights gained from the present work would be able to widen the industrial applications of SDAG and its fractions.

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