ISOTHERMAL CRYSTALLIZATION PROPERTIES OF RBD-PALM OIL AS MEASURED BY DSC AND OSCILLATORY RHEOMETRY

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ABSTRACT

In this research, the crystallization properties of refined bleached and deodorized (RBD) palm oil under isothermal conditions were investigated. A good quality of RBD palm oil (showed by IV, FFA content, PV, and AV with the values of 52.14, 0.06%, 1.39 meq/kg, and 0.88, respectively) was used in this research. In general, RBD palm oil demonstrated a two-step crystallization process at the isothermal crystallization temperatures/$T_{Cr}T_{Cr}$ *of* 18^{o}*C,* 20^{o}*C and* 22^{o}*C. From differential scanning calorimetry (DSC) measurement of RBD palm oil, the melting heats/*$\Delta H_{melting}\Delta H_{melting}$ *showed decrement values (33.84 J/g, 26.83 J/g and 22.47 J/g) but the peaks of the corresponding endotherm/*T_MT_M *showed increment values (*33.52^{o}*C,* 34.72^{o}*C and* 36.44^{o}*C) when both were correlated to the increased values of isothermal crystallization temperatures/*$T_{Cr}T_{Cr}$ *(*18^{o}*C,* 20^{o}*C, and* 22^{o}*C, respectively). From rheometry analysis of RBD palm oil (at the isothermal crystallization temperature of* 18^{o}*C), the rheograms gave the values of the phase shift angle (*$\delta\delta$*), storage modulus (*$G^{'}G^{'}$*) and complex modulus (*$|G^*||G^*|$*) at the end of crystallization process as follows:* 24^{o}*, 11.36 kPa, and 39 kPa. The overlay of* $\delta\delta$ *and* $G^{'}G^{'}$ *gave* $|G^*|_{crossover}|G^*|_{crossover}$ *with the value of 49.6 Pa at the isothermal time of 11.7 minute.*

Keywords: *Palm oil, crystallization, isothermal, DSC and rheometry*

INTRODUCTION

Palm oil is one of the most important sources of edible oils and fats in the world, just the second after soybean as the biggest turnout of vegetable oils. In tropical countries, palm oil will exist as a mixture of solid and liquid forms since the oil naturally contains both high and low melting triacylglycerols approximately at an equal ratio. According to Kellens (1991), the high and low melting triacylglycerols can be separated by a partial crystallization process. High melting triacylglycerols will crystallize into solid fraction as stearin and low melting triacylglycerols will remain in liquid fraction as olein (Zaliha *et al.*, 2004). The success of that separation depends on the appropriateness of the crystallization condition.

Crystallization also takes important functions to product qualities of fats and oils, especially the texture, consistency, emulsion stability and other physical properties (Foubert, 2003). Fat crystallization can be divided into three stages, namely primary crystallization, microstructural development, and macroscopic formation. Moreover, the primary crystallization has three distinctive steps as follows: super cooling of the melt, formation of crystal nuclei and crystal growth. All of those stages are influenced by chemical compositions (*i.e.* triacylglycerols, minor components) and processing conditions (*i.e.* temperature, time, shear) (Marangoni and Hartel, 1998).

The phase behavior of palm oil is considered to be extremely complex due to the presence of the polymorphism on the solid phase (Berger, 1996). Palm oil may crystallize into three basic polymorphic forms, namely α, $\beta'\beta'$ and β polymorphs. The $\beta'\beta'$ form seems to be stabilized in crystallized palm oil. This stabilization would be related to the palmitic acid content of the oil as well as the molecular structure and the stereochemical distribution of triacylglycerols in palm oil in which they hinder the transformation from $\beta'\beta'$ form to the β form (Sato, 1999).

The aim of this research was to investigate the crystallization properties of triacylglycerols in refined bleached and deodorized (RBD) palm oil under isothermal conditions. The analytical methodologies used to evaluate triacylglycerols crystallization in RBD palm oil were differential scanning calorimetry (DSC) and oscillatory rheological measurement. The parameters obtained from the measurements (*e.g.* the heat of melting/$\Delta H_M \Delta H_M$, complex modulus/$G^* G^*$ and phase shift angle/δ) were used to describe triacylglycerols crystallization, particularly at the stages of the onset on nucleation and crystal growth.

MATERIALS AND METHODS

Materials

The refined palm oil used as the sample on this research is termed as RBD (refined, bleached and deodorized) palm oil. The oil has an origin from Sumatra plantation. All chemicals were either of analytical or high-performance liquid chromatography (HPLC) grades.

Chemical Characterization

Determination of triacylglycerol (TAG) distribution. The distribution of the triacylglycerols was determined by HPLC, according to AOCS Official Method Ce 5b–89 (AOCS, 1996), with a differential refractometer as a detector. Minor practical adjustments to the flow rate (1.2 ml/min) and mobile phase composition (63.5 % acetone and 36.5 % acetonitrile mixture) were made in order to improve TAG separation. The column and detector temperatures were 40°C. The HPLC system consisted of Waters 515 HPLC pump, Waters 2414 refractive index detector, Waters 717 HPLC with auto sampler unit and Waters silica column.

Iodine value (IV). The IVs of the RBD palm oil was determined using the AOCS officially recommended method Cd 1b–87 (AOCS, 1996).

Free fatty acids content (FFA). The amount of free fatty acids was determined by dissolving a known quantity of fat in ethanol and subsequent titration with aqueous potassium hydroxide as described on the AOCS officially recommended method Ca 5a-40 (AOCS, 1996).

Peroxide value (PV). The peroxide of the sample was determined using the acetic acid – isooctane method as described on the AOCS officially recommended procedure Cd 8-53 (AOCS, 1996).

_p-Anisidine value (AV). The p-Anisidine value was used to determine the amount of secondary oxidation products (principally 2-alkenals and 2,4-dienals) in the oil sample. The measurement was carried out using AOCS officially recommended method Cd 18-90 (AOCS, 1996).

Isothermal DSC Analysis

DSC analysis was carried out using a Q1000 DSC (TA Instruments, New Castle, USA) with a refrigerated cooling system (TA Instruments) using aluminum SFI pans. Calibration was made with indium and *n*-dodecane standards. Nitrogen was used as purge gas in order to prevent condensation in the cells. An empty aluminum SFI pan was used as reference. The sample was heated at 70 °C for 10 minutes to erase all crystal memory, then fast cooled to the isothermal crystallization temperature ($T_{Cr} T_{Cr}$: 18 °C; 20 °C and 22 °C) for 60 minutes at a cooling rate of 10°C/min.

After completion of the crystallization exotherm (heat capacity returned to the baseline), the melting thermogram was determined at a heating rate of 10°C/min. For each T_{Cr} T_{Cr}, the heat of melting ($\Delta H_M \Delta H_M$) and the temperature at the peak ($T_M T_M$) of the corresponding endotherm were calculated using the first derivative of the heat capacity. T_M T_M was considered the apparent melting temperature of the polymorph crystallized from the melt of the oil sample at a particular $T_{Cr} T_{Cr}$.

Oscillatory Rheometry Measurements

AR-2000 controlled stress rheometer was used to perform the small deformation oscillatory measurement using starch pasting cell (SPC). A gap of 5500 µm was used for all measurements to minimize the risk of clogging of the oil sample. A temperature program was applied as follows: 1) the oil sample was melted at 70°C to erase all crystal memory and kept isothermally for 10 minutes; 2) cooling at 10°C/min to the desired crystallization temperature/$T_{Cr} T_{Cr}$; 3) oscillatory time sweep at the achieved isothermal crystallization temperature.

The linear viscoelastic region (LVR) was employed to the system during cooling and isothermal crystallization stage. A constant frequency of 1 Hz and a very low strain of 0.45% were used in all oscillatory measurements to ensure that the crystallization process was performed within the viscoelastic region without any noticeable disturbances.

The rheograms were obtained by plotting the mean values of storage modulus ($G' G'$), complex modulus ($|G^*|$ $|G^*|$) and the phase shift angle (δ) as a function of isothermal crystallization time.

RESULTS AND DISCUSSION

Chemical Characterization of RBD Palm Oil

Table 1. Triacylglycerols distribution of RBD palm oil

TAG-species*	TAG-distribution (%)
DAG **	4.83 ± 0.45***)
PLL	0.94 ± 0.02
OOL	1.86 ± 0.04
POL	10.76 ± 0.20
PLP	8.45 ± 0.25
OOO	4.19 ± 0.06
POO	24.10 ± 0.39
POP	29.64 ± 0.13
PPP	5.56 ± 0.09
SOO	2.82 ± 0.01
POS	5.76 ± 0.28
PPS	1.24 ± 0.01

* M (Myristic), P (Palmitic), S (Stearic), O (Oleic) and L (Linoleic).

** Diacylglycerols (DAG) was calculated using the similar response factor of those TAG.

*** Each value in the table represents the mean ± standard deviation from three analyses and three replications.

RBD palm oil is well known having a relatively clear mixture of high and low melting triacylglycerols in addition to a small amount of minor components, such as mono and diacylglycerols. The chemical composition of RBD palm oil will determine the way of the oil gone to the crystallization process. The triacylglycerols profile of RBD palm oil was determined by HPLC and the relative amount of each component is presented in Table 1. It was found that the three main triacylglycerols were POP (29.64 %), POO (24.1 %) and POL (10.76 %). The oil also showed the presence of a small amount of diacylglycerols (4.83 %). These results agree with the others, previously reported for RBD palm oil (Siew and Ng, 1995; deMan, 2000).

The chemical characteristics of RBD palm oil used in this study were given in Table 2. The RBD palm oil had an IV of 52.14. The oil sample was considered to have a good quality as usually shown by fresh oil. Indicators of a good quality of the oil sample were showed by low values of FFA (0.06 %), PV (1.39 meq/kg) and AV (0.88). The level of FFA indicates the amount of the acidic components in the oil. According to Sherwin (1968), the determination of FFA value by titration does not differentiate between acids formed by oxidation and those formed by hydrolysis.

Table 2. Quality characteristics of RBD palm oil

Characteristics of the oil	Value
IV (g of I2/100 g oil)	52.14 ± 0.59*
FFA content (%)	0.06 ± 0.02
PV (meq hydroperoxide/kg oil)	1.39 ± 0.23
AV	0.88 ± 0.16

* Each value in the table represents the mean ± standard deviation from three analyses and three replications.

Meanwhile, the PV and AV were used to estimate the extent of the oil deterioration caused by oxidation. Peroxides are unstable and can break down to carbonyl and aldehyde compounds giving the value of PV to rise and fall over storage. Because of this fact, AV will be necessary to be measured as supplemental information on deciding the extent of oil deterioration. Egan *et al* (1981) stated that good quality oil usually has a PV below 10 meq/kg.

In this context, it is important to point out that the chemical composition and the quality of the oil will give strong effects to its crystallization behavior. According to Siew and Ng (1999), palm oil diacylglycerols may inhibit the nucleation process and retard the crystal growth rate of palm triacylglycerols. The extent of inhibition depends on the nature of the diacylglycerols and its concentration. Wright *et al.* (2000) also pointed out that minor components (such as partial acylglycerols and free fatty acids) influence fat crystallization. For example, monoacylglycerols have been considered as an additive to improve spreadability of butter and to stabilize the preferred crystal polymorphs in chocolate products.

Isothermal Crystallization as Measured by Differential Scanning Calorimetry (DSC)

The isothermal crystallization thermogram at various crystallization temperatures ($T_{Cr}$$T_{Cr}$: 18 °C, 20 °C and 22 °C) was shown in Figure 1. At every crystallization temperature, a major peak was found in addition to a small shoulder at the beginning of crystallization time. However, the shoulders (indicated as the first peaks in the Figure 1) were not clearly observed because of the DSC limitation on the analysis running. The instrument needs to equilibrate for a new setting condition that causes a disturbance on the reading of the oil sample on that point. Since crystallization process actually has started before reaching the crystallization time, the disturbance of the instrument reading caused an imperfect curve at the beginning part of the thermogram curve.

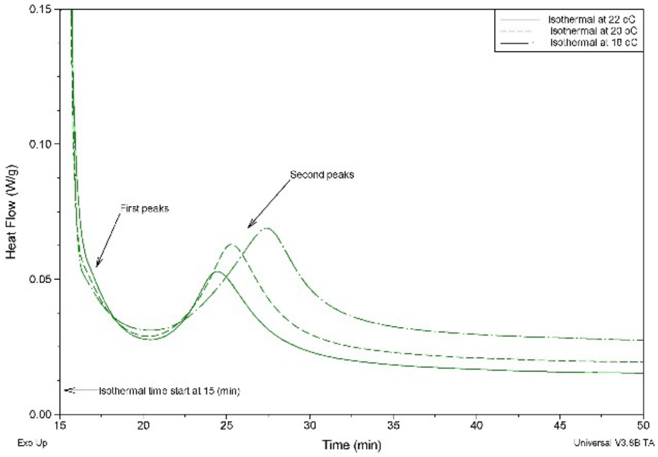

Figure 1. Isothermal crystallization of RBD palm oil

Two peaks observed in the thermogram implied that the crystallization process occurred in two steps. According to Chen *et al.* (2002), lower degrees of supercooling of palm oil will give a one-step crystallization process while at higher degrees of supercooling can change the crystallization behavior of palm oil into a two-step processes. Analogously with cocoa butter, the first exotherm was assigned to the α polymorph while the second peak was assigned to the β' β' polymorph. Once the initial β' β' crystals were developed via a polymorphic transition from α crystal, additional β' β' crystallization might occur directly from the melt through a facilitated nucleation mechanism. This mechanism occurred giving the β' β' template previously developed in the system (van Malssen *et al.*, 1999; Toro-Vazquez *et al.*, 2004).

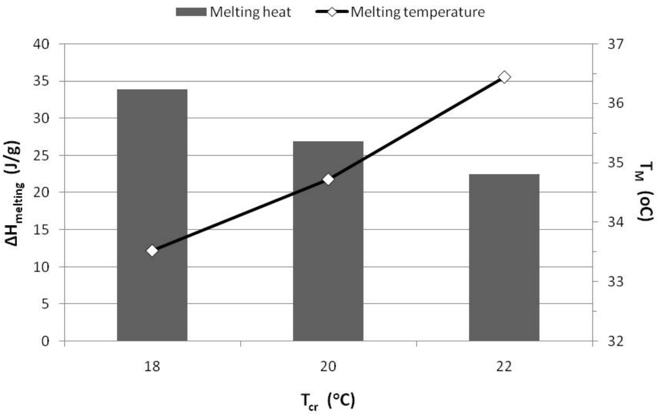

Figure 2. Relationship between the isothermal crystallization temperature (T_{Cr} T_{Cr}) with the melting heat ($\Delta H_{melting}$ $\Delta H_{melting}$) and melting temperature (T_M T_M)

The behavior of the melting heat ($\Delta H_{melting}$ $\Delta H_{melting}$) and the temperature at the peak of the corresponding endotherm (T_M T_M) for RBD palm oil as function of isothermal crystallization temperature (T_{Cr} T_{Cr}) was shown in Figure 2. There was an inverse relationship between the isothermal crystallization time and the corresponding melting heat. At increased isothermal crystallization temperatures (18 ^{o}C, 20 ^{o}C and 22 ^{o}C), the thermograms gave the decrement values of melting heats (33.84 J/g, 26.83 J/g and 22.47 J/g, respectively) and the increment values of the peak of the corresponding endotherm (33.52 ^{o}C, 34.72 ^{o}C and 36.44 ^{o}C, respectively). This effect was associated with the higher amount of solid phase developed in the melt through triacylglycerols crystallization as the degree of supercooling increased. The lower crystallization temperature will assist the medium-high melting triacylglycerols (*i.e.* POO and POL) to be present more in the solid phase in addition to the high melting triacylglycerols (*i.e.* POP and PLP). On contrary, as the solid phase contained more unsaturated triacylglycerols, the melting temperatures of the solid phase had lower values when the crystallization temperatures decreased.

Isothermal Crystallization as Measured by Oscillatory Rheometry

Rheology is the study of deformation and flow of materials. According to Toro-Vazquez *et al.* (2000), oscillatory rheology can be used to study fat crystallization by following the storage modulus, loss modulus, complex modulus or phase angle as a function of isothermal time at a desired crystallization temperature. Figure 3 showed the storage modulus (G' G') as a function of time for the crystallization of RBD palm oil at different crystallization temperature (T_{Cr} T_{Cr}: 18 ^{o}C, 20 ^{o}C and 22 ^{o}C).

Figure 3 showed at all isothermal crystallization temperatures gave an increase of storage modulus (G' G') with

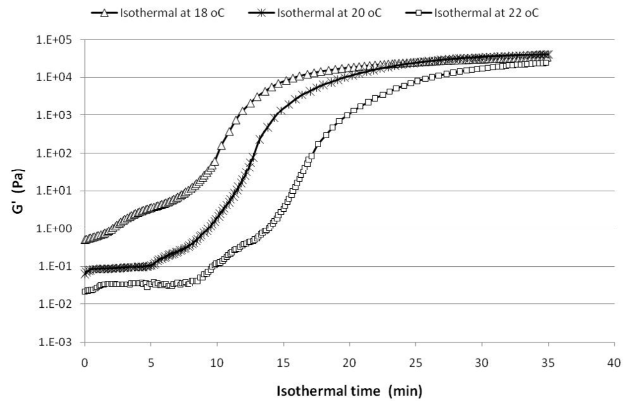

Figure 3. Storage modulus (G' G') rheogram for the crystallization of RBD palm oil

the advance of crystallization time until reaching a plateau at the end of crystallization process. The increases of the storage modulus ($G'G'$) as a function of crystallization time under isothermal conditions were associated with the crystal mass development. After nucleation process, the crystals grew up to a certain size and started to build up a network formation. The system then turned into a more elastic liquid. A higher value of storage modulus ($G'G'$) indicated that the sample behaved more elastically (solid-like).

At the beginning of isothermal time (for all crystallization temperature/$T_{Cr}T_{Cr}$), the rheogram showed a small bend before the curves went to a sigmoid increase of storage modulus ($G'G'$). This result strengthened that of isothermal DSC measurement (as shown in Figure 1) showing a two-step crystallization process. Moreover, all curves gave about similar values of storage modulus ($G'G'$) for all crystallization temperature/$T_{Cr}T_{Cr}$ at 11.36 kPa at the end of crystallization process. This indicated that the difference of those crystallization temperature/$T_{Cr}T_{Cr}$ did not affect the properties of crystallized system. Toro-Vasquez *et al.* (2004) also pointed out that storage modulus ($G'G'$) can be associated with three-dimensional organization of the triacylglycerols crystal network.

An overlay of the phase shift angle (δ) and complex modulus ($|G^*||G^*|$) as a function of time for RBD palm oil at crystallization temperature 18 °C is depicted in Figure 4. Phase shift angle (δ) is used to measure the relative degree of viscoelasticity of a material. An ideally elastic material will have a phase angle of 0° and an ideally viscous material will have a phase angle of 90°, while a viscoelastic material will have a phase angle somewhere in between. Once triacylglycerols achieve the lamellar organization that develop a nucleus, a drastic decrease of phase shift angle (δ) will follow the phase change from liquid to solid. Eventually, the phase shift angle (δ) will become zero when the system becomes fully crystallized. Moreover, the heat released during the crystal growth is shown as a peak in the δ rheogram (Toro-Vazquez *et al.*, 2002).

Figure 4 showed a sharp decrease of phase shift angle from 88° (at the isothermal time of at about 4 minute) to a base line of 24°. It was hypothesized that most of the liquid material crystallized during this decrease of phase shift angle from 88° to 24°. This result was in accordance with that of the DSC measurement (as shown in Figure 1) where the major crystallization process was shown by one major peak after the isothermal crystallization time of 5 minute. The drastic decrease of phase shift angle (δ) could be related to the formation of $\beta'\beta'$ crystals which can be directly formed from the melt and through polymorphic transition from α to $\beta'\beta'$. After the crystallization process finished, the phase shift angle (δ) still gradually decreased due to ongoing aggregation of crystals and further strengthening of the network.

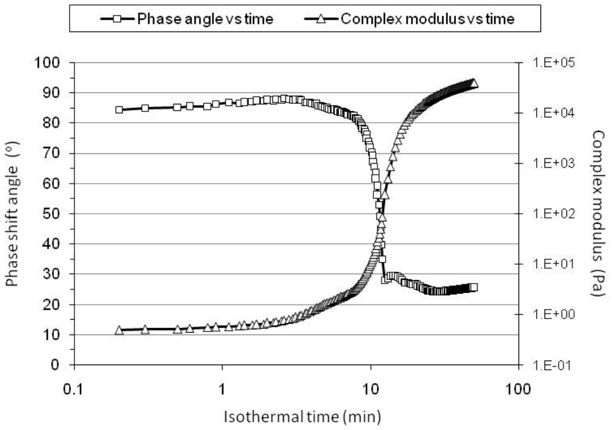

Figure 4. Phase shift angle (δ) and complex modulus ($|G^*||G^*|$) of isothermal crystallization RBD palm oil at 18 °C

The complex modulus ($|G^*||G^*|$) as a function of isothermal time is also depicted in Figure 4. At the initial isothermal crystallization time, the complex modulus ($|G^*||G^*|$) had a value of approximately 0.5 Pa which indicated the liquid behavior of the oil sample. Then, a sigmoid increase of the complex modulus ($|G^*||G^*|$) was noticed after 4 minutes of the isothermal time. At this point onward, most of the liquid material would crystallize. Eventually, the complex modulus ($|G^*||G^*|$) reached a plateau with the value of approximately 39 kPa. Kloek *et al.* (2000) stated that further strengthening of the network through ongoing crystallization or aggregation will cause a slightly increased trend of the complex modulus.

The overlay of phase shift angle (δ) and complex modulus ($|G^*||G^*|$) curves (as shown in Figure 4) gave a parameter on the intersection point, denoted as $|G^*|_{crossover}|G^*|_{crossover}$. The cross over point can be considered as the point at which the phase angle value is 45° and this implies that the value of viscous modulus and elastic modulus are equal ($G''G''=G'G'$). The value of $|G^*|_{crossover}|G^*|_{crossover}$ reflects the amount, structure and strength of the crystal network formed at $G''G''=G'G'$. For isothermal crystallization of RBD palm oil at 18°C, the $|G^*|_{crossover}|G^*|_{crossover}$ had a value of 49.6 Pa at isothermal time 11.7 minute.

CONCLUSIONS

Both calorimetry (using isothermal DSC measurement) and rheometry (using oscillatory rheology measurement) can be utilized to evaluate the crystallization behavior of RBD palm oil. The thermogram gave two peaks on the exothermic curves while the rheogram showed a sigmoidal curve in addition to a moderate bend at the beginning of the isothermal crystallization time. Both measurements showed that RBD palm oil had a two-step crystallization process at the isothermal crystallization temperatures of 18 °C, 20 °C and 22 °C. The higher degree of supercooling assisted the medium-high melting triacylglycerols (*i.e.* POO and POL) to be present more in the solid phase in addition to the high melting triacylglycerols (*i.e.* POP and PLP) giving a lower melting temperature. Crystallization of RBD palm oil at isothermal temperatures of 18 °C, 20 °C and 22 °C gave melting temperatures of 33.52 °C, 34.72 °C and 36.44 °C, respectively.

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