

Crystal habit during crystallization of RBDPO with the addition of Diacylglycerol (DAG): effect of time and percentage of DAG added

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Abstract

Diacylglycerol at 1 or 6% was added into refined bleached and deodorized palm oil (RBDPO) and crystallized from the melt in a thermally controlled water bath at 22°C for 90 min. Slurries were withdrawn after 5, 15, 30, 60 and 90 min of crystallization for solid fat content (SFC) and crystal morphology studies. Crystallization was also performed in a similar manner using a Labmax reactor connected to a FBRM detector to obtain the information on crystal count and size distribution during crystallization. SFC of the slurries increased with increase in crystallization time up to a certain level followed by a plateau. SFC of RBDPO added with DAG was also higher with the increase in percentage of DAG added and no induction time was observed to initiate crystallization in RBDPO added with DAG. The addition of DAG caused rapid crystallization of RBDPO as observed by enhance nucleation and larger crystal size with increase in the percentages of DAG added.

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Introduction

Refined palm oil consists of 80 to 90% triacylglycerols (TAG), while the diacylglycerol (DAG) content vary from 7 to 8% and those with free fatty acids below 2% may contain 4 to 5% DAG (Okiy, 1978; Jacobsberg and Jacqmain, 1976; Jacobsberg and Oh, 1976). Palm oil fractions; olein and stearin were reported to comprise of 6.8 and 5.8% DAG, respectively (Goh and Timms, 1985). The main DAG in palm oil are dipalmitoylglycerol (PP), palmitoyloleoylglycerol (PO) and dioleoylglycerol (OO) (Siew and Ng, 1995).

DAG have been shown to exert several effects on the crystallization behavior of fats and oils. Depending on the type of DAG used, it may either enhance or retard crystallization. A high melting DAG such as dipalmitin has been shown to enhance nucleation (Wright *et al.*, 2000). In its presence, dipalmitin may likely act as a seed crystallizing out before the TAG and subsequently inducing nucleation and crystal growth whereas a DAG that is complementary to the TAG may cause the DAG to co-crystallize with the TAG, be incorporated into the embryos or growing crystals and subsequently retard crystallization (Wright *et al.*, 2000). In the studies of Siew and Ng (1996), the addition of 0.5% 1, 3 dipalmitin (PP) caused a rapid crystallization of palm olein whereas palmitoyloleoylglycerol (PO) retard crystallization while dioleoylglycerol (OO) had no effect. In another study, dilaurin has been shown to retard nucleation

of coconut oil whereas diolein had no effect (Gordon and Rahman, 1991). DAG in different isomeric forms such as 1, 3 DAG has been shown to have greater effect than 1, 2 DAG (Smith and Povey, 1997). The presence of DAG also delayed the polymorphic transition in palm oil, coberine fat and sal fat (Okiy, 1978; Siew and Ng, 1999; Cebula and Smith, 1992; Reddy and Prabhakar, 1986). The effect of DAG was said to be more pronounced at higher percentage of DAG and higher heating rate (Reddy and Prabhakar, 1986). Furthermore, the addition of DAG caused a rapid crystallization of olein with higher degree of unsaturation (Siew and Ng, 1996). It also reduced crystal growth rate (Wright *et al.*, 2000; Smith and Povey, 1997; Okiy, 1978). This study investigated the effect of time and percentage of DAG addition on the solid fat content (SFC), crystal morphology and chord length distribution of RBDPO added with 1 or 6% DAG.

Materials and Method

Materials

Refined Bleached and Deodorized Palm Oil (RBDPO) with iodine value of 53 was obtained from Golden Jomalina Food Industries Sdn. Bhd (Sijangkang, Selangor Malaysia).

Preparation of diacylglycerol (DAG)

DAG in the form of dipalmitin was prepared by glycerolysis in the presence of solvent (isooctane)

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and Lipozyme RM 1M lipase according to Normah *et al.* (2011). Sixty four grams of palmitic acid was placed into a beaker and 100 ml iso-octane was added. The beaker and its content was then placed in a thermally controlled water bath (Haake, Germany) preset at 65°C and left to completely melt the palmitic acid. 11.5 g glycerol anhydrous (2:1 molar ratio of palmitic acid to glycerol), 4% Lipozyme RM 1M lipase (based on the total weight of palmitic acid and glycerol) and 10% molecular sieves (based on the total weight of palmitic acid and glycerol) were added. The mixture was continuously stirred using a stirring motor (Model IKA RW11, Staufen, Germany) for 3.5 hr followed by vacuum filtration through Whatman filter paper #1. The vacuum process was continued until the collected filtrate in the conical flask was dried to evaporate as much solvent as possible. The filtrate was further air dried and then subjected to neutralization with sodium hydroxide according to PORIM (1995) to remove the unreacted free fatty acids and monopalmitin. This was followed by crystallization at 50°C for 1 hr.

Crystallization process

Crystallization process of RBDPO was performed as previously mentioned by Normah *et al.* (2013). However, RBDPO was added with 1 or 6% diacylglycerol (DAG) prior to crystallization. RBDPO containing 1 or 6% DAG which was placed in a beaker was initially heated in a thermally controlled water bath for 30 min at 70°C to totally melt the oil. The temperature was then reduced to 30°C within one hour followed by reducing to crystallization temperature of 22°C within 30 min. Once the oil reached the desired temperature (22°C), the crystallization process was further proceed for 90 min. The beaker content was constantly stirred at 90 rpm throughout the process using a stirring motor attached with two blades paddle propeller (Model IKA RW11, Staufen, Germany).

Determination of solid fat content (%)

Solid fat content (SFC) was determined according to the PORIM parallel test method (PORIM, 1995) using pulsed nuclear magnetic resonance (pNMR) spectrometry set at 22°C (Bruker Minispec P20:20 Mhz, Karlsruhe, Germany). RBDPO slurries formed during the crystallization process were withdrawn and filled into the NMR tube up to 3 cm height. The slurries which were withdrawn every 5 min interval during the first half an hour and then every 10 min were immediately measured for the SFC.

SFC measurement was done until the end of the day and left overnight before the last measurement was recorded in the following day.

Crystal size distribution

Determination of chord length distribution which reflects the crystal size distribution was carried out using Labmax reactor (Mettler Toledo, Schwerzenbach, Switzerland) equipped with Lasentec D600L Focused Beam Reflectance Measurement (FBRM) probe (Lasentec, Richmond, WA, USA). FBRM records the chord length distribution in terms of the number of counts per second for different crystal size classes. Melted RBDPO (400 ml) was poured into the Labmax reaction vessel consisting of four openings for pouring of sample, insertion of thermometer, FBRM probe and stirrer. The temperature of the Labmax reactor was then reduced to 30°C within 1hr and then further reduced to the measuring temperature (22°C). The total crystallization time at 22°C was 90 min. During the run, samples were continuously stirred by a four blade impeller set at 100rpm. Data were collected using 90 log-channel over the range of 1-1000 µm with scanning speed set at 2 m/s. Chord length distribution was measured every 15 sec and data were recorded every 5, 15, 30, 60 and 90 min. Crystals count that fell within the crystal size classes of 1 to 5 µm, 10 to 23 µm, 29 to 86 µm and 100 to 251 µm were recorded.

Crystal morphology

Crystal morphology (size and shape) of RBDPO slurries collected during the crystallization process was obtained using a polarized light microscope (Leica DMLP) equipped with a temperature controlled stage (Linkam TP94 and LNP). Images were recorded using the Leica Qwin V3 imaging system (Cambridge, UK). The temperature of the stage was preset to simulate the crystallization temperature (22°C). Zero time denotes the time when the temperature of the water bath reached 22°C. Samples of slurries were withdrawn at 5, 15, 30, 60 and 90 min of crystallization and placed onto a slide which was then covered with a cover slip. Photographs of the crystals were taken at the magnification of 200x.

Statistical analysis

Data were analysed using the Analysis of Variance (ANOVA) to determine significance at 5% level. Duncan Multiple Range Test was used to identify differences between means. The software used was the SAS system for windows release 6.12

SAS Institute Inc. (SAS, 1989).

Results and Discussion

Solid fat content (SFC) of RBDPO slurry: effect of time

Figure 1 shows that SFC of the slurries produced with the addition of 0, 1 or 6% DAG increased with increase in crystallization time up to a certain period where thereafter the SFC were constant. The SFC curves started to plateau at approximately 110, 180 and 80 min of crystallization at 0, 1 and 6% DAG addition, respectively, indicating complete crystallization has occurred within these times. For the 6% DAG addition, SFC fluctuated especially towards the end of the crystallization period which could probably due to the increase in viscosity of the slurry.

Solid fat content (SFC) of Slurry: effect of percentage of DAG added

SFC of slurries produced with the addition of DAG were higher than those without the addition of DAG. Increase in the percentage of DAG added resulted in increase in SFC (Figure 1). This shows that the added DAG had a promoting effect on the crystallization of RBDPO as reflected by the formation of higher amounts of solid. Five minutes induction time was observed for slurries produced without DAG addition but no induction time was observed for RBDPO with DAG addition, indicating that crystallization occurred instantaneously in slurries with DAG addition. According to Campos *et al.* (2002), induction time is inversely proportional to the rate of nucleation, thus shorter induction time indicates higher nucleation rate. Since no induction time was observed for slurries produced with DAG addition, this observation suggested that DAG addition enhanced higher nucleation rate and initiate earlier crystallization of RBDPO. Additionally, the DAG added were enriched in palmitic acid and this may encourage the interaction of the DAG with triacylglycerols (TAG) within RBDPO which also contain palmitic acid allowing the DAG to co-crystallize with the TAG. Hence, DAG addition possibly encouraged higher nucleation rate as illustrated by higher SFC. This is consistent with the formation of a large amount of fine crystals at higher percentages of DAG addition (Figure 2A and B, Figure 3A and B).

Chord length distribution: effect of time

Chord length distribution of RBDPO added with 1% DAG and crystallized at 22°C is shown

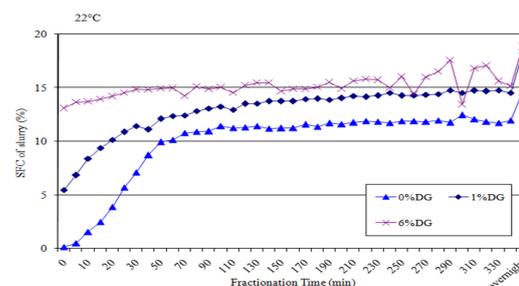


Figure 1. SFC (%) of RBDPO slurries obtained during the crystallization with the addition of 1 or 6% DAG at 22°C.

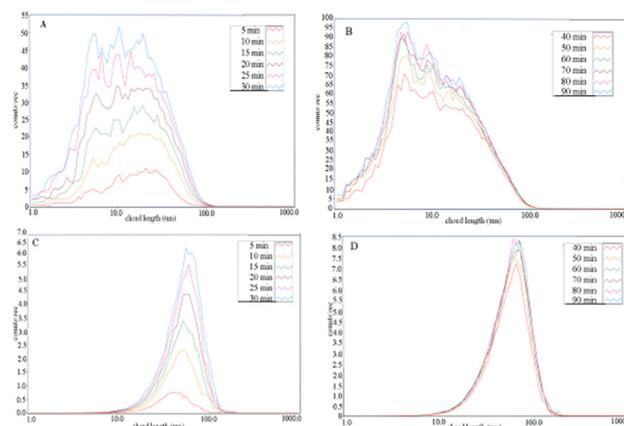


Figure 2. Chord length (μm) distribution of RBDPO added with 1% DAG and crystallized for 90 min at 22°C (1/Lth wt; A, B) and (Square wt; C, D).

in Figure 2. 1/Lth wt represents fine crystals while Sq wt represents large crystals. Addition of 1% DAG resulted in a broad distribution of size with peaks ranging from 5 to 50 μm for fine crystals (Figure 2A). A gradual increase in count with time was observed during the first 30 min of crystallization. At 40 to 90 min of crystallization, the counts continuously increase at a slower rate (Figure 2B). Large crystals (29 to 86 μm) also increased gradually during the first 30 min of crystallization (Figure 2C). However, the count was low (<6.5 counts/sec) with narrower size distribution. A very slight shift to a larger size range was observed to occur with time, indicating crystal growth (Figures 2C and D). This could be due to crystal agglomeration which is in line with microscopic observation (Figure 4A). At 6% DAG addition, counts of fine crystals increased very rapidly while counts of large crystals were constant throughout the 90 min of crystallization (Figure 3).

Chord length distribution: effect of percentage of DAG added

The approximate peak sizes of the large crystals for crystallization at 22°C with 1 and 6% DAG were respectively, 80 and 110 μm during the first 30 min of crystallization (Figure 2C and 3C) and 85 and 110 μm during the 40 to 90 min of crystallization period

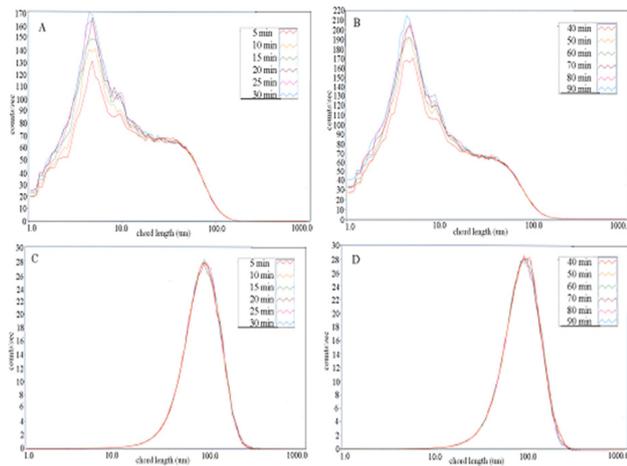


Figure 3. Chord length (μm) distribution of RBDPO added with 6% DAG and crystallized for 90 min at 22°C (1/Lth wt; A, B) and (Square wt; C, D).

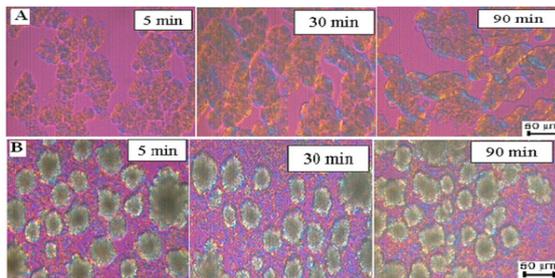


Figure 4. Light microscope photos of crystals formed after RBDPO added with 1% DAG (A) or 6% DAG (B) were crystallized at 22°C for 5, 30 and 90 min crystallization.

(Figure 2D and 3D). This suggested that crystals were larger with increase in the percentages of DAG addition. The slurries were dominated by large crystals (29 to 86 μm) at all percentages of DAG addition (Figure 5).

Crystal morphology: effect of time

Figure 4 shows the microscopic photos of crystals in the slurries of RBDPO with the addition of 1 and 6% DAG during crystallization at 22°C . Not much difference in terms of density and crystal size were observed with the increase in crystallization time at all percentages of DAG addition.

Crystal morphology: effect of percentage of DAG added

Addition of 1% DAG resulted in the formation of large-sized crystal agglomerates (Figure 4A). During the crystallization process, the slurries formed with the addition of 1% DAG were observed to be more liquid compared to those with 6% DAG addition. Crystal agglomeration has been shown to occur during a slow crystallization process (Campos *et al.*, 2002). In this study, large-sized agglomerates were observed in slurries with 1% DAG addition, indicating that crystallization was comparatively slower than those

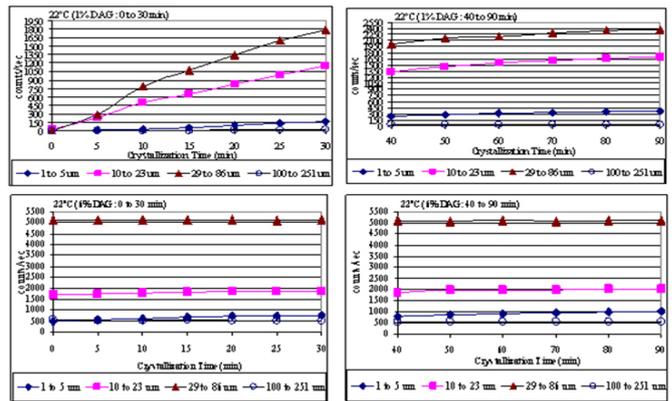


Figure 5. Crystal population (counts/sec) in RBDPO added with 1 or 6% DAG and crystallized at 22°C (0 to 30 min and 40 to 90 min).

in the presence of 6% DAG. Crystals were seen to be larger at 6% DAG addition. This result is in-line with the result for chord length distribution. Crystal growth could be promoted by surface nucleation or movement of α crystals leading to the formation of aggregates (Kawamura, 1979; Okiy, 1978). Probably, the addition of DAG, especially at 6%, promotes surface nucleation of the crystals whereas the addition of 1% DAG accelerated the movement of α crystals.

Microscopic image also showed that the large crystals in 6% DAG addition were surrounded by numerous small crystals (Figure 4B). However, this was not observed with the 1% DAG addition where the background was clear. This suggested that the addition of DAG at high percentages (6%) also enhanced nucleation of RBDPO. This is in-line with the previous observation where the addition of 5% 1,3-dipalmitin caused instantaneous nucleation of palm TAG oil (Siew and Ng, 1999). At high percentage of DAG addition, the high melting DAG added may act as seeds that crystallized out before the TAG, thus inducing faster nucleation and crystal growth. At 1% DAG addition the increased in growth rate observed was most probably due to aggregation of the existing smaller crystals, as can be seen from the formation of crystal agglomerates. At 6% DAG addition, growth was more associated with the increase in size of the individual crystals. The formation of larger crystal size was similar to the observations by Herrera and Hartel (2000). In their studies the presence of higher ratio of high melting fraction in the blend of high melting : medium melting fraction of milk fat resulted in the development of larger crystals and denser background.

Conclusion

The addition of DAG in the form of dipalmitin caused rapid crystallization of RBDPO where

crystallization increased with increase in the percentages of DAG added. The presence of DAG enhanced nucleation and probably even crystal growth, especially at 6% DAG addition as evidence by microscopic observation, chord length distribution and higher amount of solids formed.

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