Thermal and Structural Behavior of Palm Oil. Influence of Cooling Rate on Fat Crystallization

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Summary: The effects of scanning rates ($q = -0.5\ ^\circ$C/min to $-50\ ^\circ$C/min) on the formation of the different phases occurring at low temperature of a palm oil are investigated by means of calorimetry and optical analysis. It is demonstrated that the cooling rates changed the polymorphism of triacylglycerols (TAGs). The centrifugation is used to separate the two fractions (olein and stearin) of palm oil. We show whereas the rate of centrifugation the separation is not effective. We obtained two fractions, a liquid fraction and a solid fraction which contains some liquid TAGs. It is observed that the solid fraction of palm oil is more sensitive to the effects of the cooling rates. By changing the cooling rate q, it appears threshold behaviour for $q = -3\ ^\circ$C/min dividing the data discussion in two parts: for slow cooling rate and for fast cooling rate. At slow cooling rates, TAGs had more time to interact. Contrary at fast cooling rate, TAGs have not the time to be reorganised in more stable conformation. Micrographs revealed that the types of crystals observed were spherolites but some variations in crystal size appeared with the variation of cooling rate.

Keywords: cooling rate; differential scanning calorimetry; fat crystallization; microstructure; palm oil

Introduction

It is established that melting and crystallization behaviours of edible oils and fats are two of the most important properties for functionality in many prepared food products. These thermal properties are counterparts of the triacylglycerol (TAGs) profile of edible oils and fats.[1] In this field, the palm oil is used as cooking oil, to make margarine and is component of many processed foods. An edible oils and fats can typically contain more than one hundred of different mainly TAGs.[2]

To obtain a given texture, oil can be crystallized.[2] It appears that crystal size can be considered as a key of flow properties with a direct influence on sensory perception.[3] For some case, the rate of crystallization of fat is very important, this is the case of chocolates and confectionary coatings, dairy products such as butter and cream and vegetable spreads.[4]

The crystalline state differs from the amorphous state as a result of the regularity of molecular arrangements.[5] The crystallization process consists of two steps: nucleation and crystal growth. Nucleation can be described as a process in which molecules come into contact, orient and interact to form highly ordered structures, called nuclei. Crystal growth is the enlargement of these nuclei. Crystallization of liquid oil results in a volume contraction and a positive (exothermic) heat effect.

Fat crystallization behaviours in refined oil were studied by thermal analysis,

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yielding products from different cooling rate. The effects of thermal process were evaluated by differential scanning calorimetry (DSC) and optical thermal analysis (OTA). It is well known that crystallization kinetics can be easily analysed by using calorimetric measurements. Ferrari et al. [6] showed the practical utilization of calorimetry in edible oil study by good DSC curves reproducibility and large agreement on the method at scientific and commercial level. Differential scanning calorimetry is a useful tool for determining the temperature of final melting and initial crystallization of fat, as well as for following polymorphic evolutions.

The objective of this study was to investigate the thermal properties of the fat in palm oil as a function of temperature during thermal process. Differential scanning calorimetry was used to characterize in situ the crystallization and melting of TAGs molecules. The melting properties and polymorphic evolutions of fats were characterized and the solid fat content was determined after different cooling rates. The microstructures of crystals were deduced from image analysis obtained by optical thermal analysis.

Materials and Methods

Industrial refined palm oil was used in these experiments without further purification. The main characteristics of the sample were acidity value equal 0.1 mg KOH/g and peroxide value equal 0.4 meq O_2/kg. The sample was sealed in clear glass jar and stored in dark at the laboratory room temperature of $22 \pm 3\,^{\circ}C$.

Centrifugation was carried out by placing 10 g of refined palm oil into a plastic tube (Ø 2 cm) and centrifuging it at three different rates: $2.6 \cdot 10^3$ g, $2.1 \cdot 10^4$ g, and $5.4 \cdot 10^5$ g for 5min at $\approx 22\,^{\circ}C$ (room temperature). This different rates are obtained using three kinds of centrifuge model, a Biofuge primoR centrifuge Heraus (France – Illange (57)) for $2.6 \cdot 10^3$ g, a sigma model 3-12 centrifuge Bioblock Scientific (France – Illkirch (67)) for $2.1 \cdot 10^4$ g, and a Beckman model TL-100 ultracentrifuge (USA – Fullerton (CA)) for $5.4 \cdot 10^5$ g. Three different rates were used to optimize the separation. Centrifugation of the sample allowed the upper liquid phase to separate from the lower crystallized phase.

Calorimetric studies were performed with a differential scanning calorimetry (DSC Q100, TA Instrument, USA - New Castel (DL)). The purge gas flow was dry nitrogen (50 mL/min). Calibration of the calorimeter was obtained from the measurement of the enthalpy (ΔHm) and the onset temperature (MP) of fusion of Indium (MP 156.6 °C; ΔHm 28.45 J/g). Samples of 7–10 mg were weighed into aluminium pans and none hermetically sealed. The temperature range scanned was −80 °C to 80 °C.

The optical thermal analysis (OTA) used for the in situ observation of the transitions was developed by Normalab, France –Lintot (76).[7] This equipment was used in this work as a hit stage microscope. The temperature range scanned was −40 °C to 60 °C. Controlled cooling rates of −0.5 °C/min to -50 °C/min and controlled heating rate of 10 °C/min were used. The accuracy of the temperature measurement is ± 0.5 °C in the whole temperature range scanned during the experiments. The observations were made in the reflexion and transmission mode and images have been observed during all the experiments duration. The diameters (μm) of the crystals were deduced from image analysis.

For both methods (DSC and OTA), palm oil samples were prepared as follows: each sample was first heated from room temperature (~20 °C) to 80 °C (DSC) or 60 °C (OTA) and maintained at this temperature for 1 min, in order to improve the thermal contact between the sample and the pan and to erase the palm oil history (polymorphism). The sample was then cooled at different cooling rates: −0.5 °C/min; -50 °C/min, to −80 °C (DSC) or −40 °C (OTA) and immediately reheated to 80 °C (DSC) or 60 °C (OTA) at the heating rate of 10 °C/min.

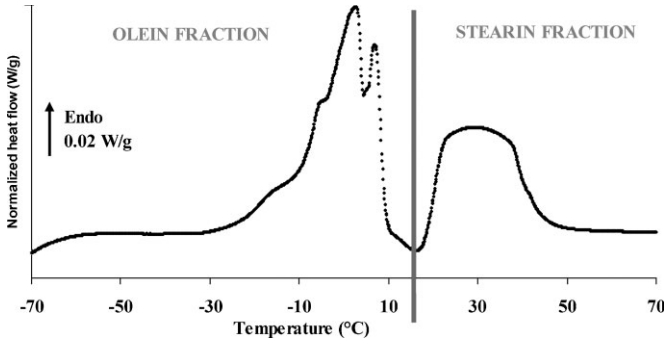

Figure 1.
Differential scanning calorimetry curve for palm oil for a scanning rate of 10 °C/min. The two characteristic peaks corresponded to endothermic transitions of the melting of olein and stearin fractions.

It is generally accepted that the partial area under the melting peak (endothermic event) is equivalent to the percentage of solid fat remaining at the selected temperature.[8] The ratio of solid to liquid fat; that is the amount of fat that is crystallized, was calculated using the DSC signal. First, the area between the DSC melting curve and the baseline was calculated to determine the partial enthalpy of melting of TAGs of palm oil. The ratio of the partial to the total enthalpy of melting was determined over the melting range of TAGs. The solid fat content within palm oil after different cooling rates was calculated as a function of temperature by constructing an integral curve from the DSC melting curves. The solid percentage is obtained by calculating the ratio of surface situated to the right of a given temperature, to the whole surface of the endotherm. The calculation of the solid fat content involved the implicit assumption that the heat of melting of TAGs was constant. In this way, the same melting enthalpy is attributed to each crystal, whatever its structure. From a more sophisticated point of view, this method give an estimated value higher than the true value as demonstrated by Santana et al.,[9] but in a first approximation we will use it because the same authors have also demonstrated that for a comparative (not absolute) studies, these errors are acceptable.

Results

Figure 1 shows a typical DSC curve obtained when heating palm oil.

Marangoni et al.;[10] Deroanne;[11] Tan et al.[12] showed that fats crystallize and melt in several steps that correspond to separated group of TAGs. The complex DSC recordings result from: the broad distribution of TAGs composition; compound crystallization meaning that the composition of the crystals is changing during melting; and the existence of a polymorphism of monotropic type for each TAGs group constituting the crystal. The palm oil curve presented in figure 1 exhibit two endothermic peaks. Generally, the two endothermic peaks corresponded to melting transitions of the olein (low-temperature endotherm; from −30 °C to 20 °C) and stearin (high-temperature endotherm, from 20 °C to 50 °C) fractions.[12] We called olein fraction the highly unsaturated TAGs and stearin fraction the highly saturated TAGs. These first results allow us to say that at room temperature, palm oil have a liquid phase and a solid phase. According to the liquid phase is the olein fraction and the solid phase is constituted of the stearin fraction. After centrifugation at room temperature of palm oil we obtain a liquid fraction and a solid fraction which constitute 50% in weight of the initial palm oil each one.

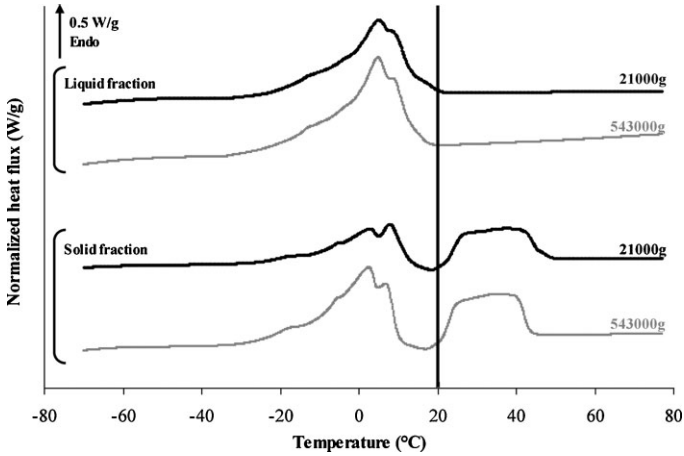

Figure 2.
Differential scanning calorimetry curves for liquid fraction and solid fraction obtained by centrifugation at 21000 g and 543000 g of palm oil at scanning rate of 10 °C/min. The liquid fraction melts below 20 °C. The solid fraction is characterised by two endothermic peaks: one below 20 °C and one above 20 °C. No effect of the rate of centrifugation is observed.

On figure 2, the DSC curves show two series of endothermic signals.

The first series corresponds to the liquid fraction which has been crystallized during the initial cooling period, while the second series corresponds to the solid fraction tested after a centrifugation at $2.1 \cdot 10^4$ g, and $5.4 \cdot 10^5$ g. The DSC curves present the same profile however centrifugation rate was different. For liquid fraction, only one endothermic peak is present and melts before 20 °C, but for solid fraction we observed two endothermic peaks, one below 20 °C and one above 20 °C. These two endothermic peaks could be explained by the presence of oil absorbed at the surface of crystals as observed by Deroanne [11]. This absorption could be due to the intersolubility of TAGs. Consequently, the endothermic peak below 20 °C is due to absorbed oil and the endothermic peak above 20 °C is due to crystalline part.

When different cooling rates are used, we obtained the DSC curves after reheating displayed on figure 3.

The arrows on theses DSC curves show the means differences observed when slow

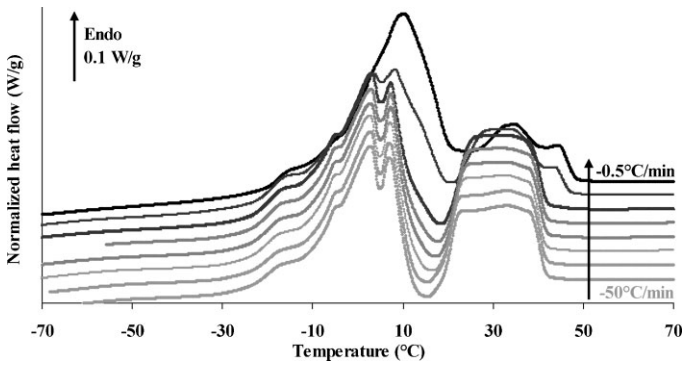

Figure 3.
Differential scanning calorimetry melting curves for palm oil obtained after different cooling rates from −0.5 °C/min to −50 °C/min. A threshold for a cooling rate of −3 °C/min is observed.

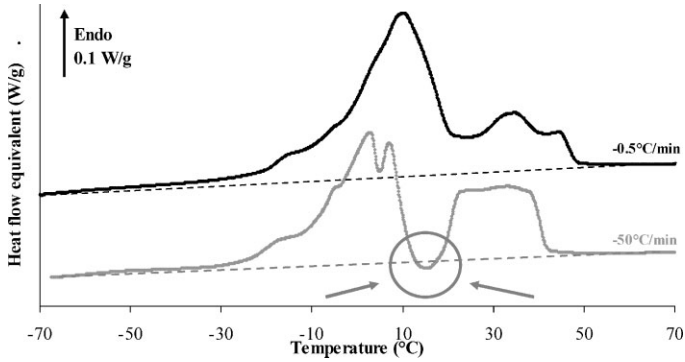

Figure 4.
Differential scanning calorimetry melting curves for palm oil for −0.5 °C/min and −50 °C/min of cooling rate. An exotherm is observed for the fast cooling rate displaying a crystalline reorganisation.

cooling rate and fast cooling rate are used. For the DSC melting curves corresponding to fast cooling rate an exotherm was clearly recorded, between 10 °C and 20 °C, between the first and the second endothermic peaks recorded on heating. The overlapped peaks display in figure 4, endothermic and exothermic, showed that several crystalline reorganisations occurred during heating.

The characteristic melting profile of palm oil after a slow cooling rate that started at ∼ −30 °C and ended at ∼ 50 °C but after a fast cooling rate that started at ∼ −30 °C and ended at ∼ 45 °C. The same thermal cycles have been performed with OTA. By this way, optical microscopy was used to observe TAGs crystals. The types of crystals observed were spherolites. Microstructural examination using optical thermal microscopy (OTA) showed variations in crystal size of fats by varying the value of the cooling rate (Figure 5).

The OTA results show that a cooling rate of -10 °C/min induced smaller crystal size than a cooling rate of −0.5 °C/min. We observed that by changing the cooling rate the number of nuclei and the crystal growth are different in rate.

Figure 6 shows the DSC curves after different cooling rate of the two fractions obtained by centrifugation: liquid fraction in A, and solid fraction in B.

For liquid fraction only some weak variations are observed in melting curves. The microstructural examination did not show variation in size and morphology. The OTA analysis for the liquid fraction shows spherolite crystals with small size. Nevertheless for solid fraction major differences in the behaviour between slow cooling rate and fast cooling rate are observed. The characteristic melting profile of solid fraction after a slow cooling rate that started at ∼ −30 °C and ended at ∼ 55 °C but after a fast cooling rate that started at ∼ −30 °C and ended at ∼ 50 °C. We noted that the gap is about 5 °C like for palm oil (Figure 3).

Discussion

The palm oil shows two melting peaks, for lower temperature we identify the olein fraction and for the higher temperature we identify the stearin fraction. Because the DSC curve of solid fraction of palm oil shows a melting peak below 20 °C, we may conclude that the result obtained by centrifugation shows that a part of the olein fraction remains by interdiffusion around crystals of the stearin fraction.

By changing the cooling rate we have noticed that slower the cooling rate, higher the melting point, and bigger the crystal size; contrary, faster the cooling rate, lower the melting point, and smaller the crystal size. According to Yano et al. [13], the basic polymorphs of TAGs are called α, β' and β which are identified by subcell structure. The β form is the most stable with a small

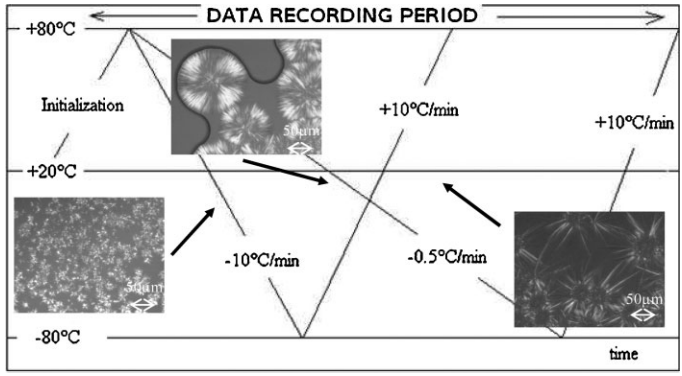

Figure 5.
Microscopic observation of palm oil crystals. The crystal size changes with the cooling rate. A slow cooling rate induces bigger crystal size. The crystal structures are evolving during the isothermal process.

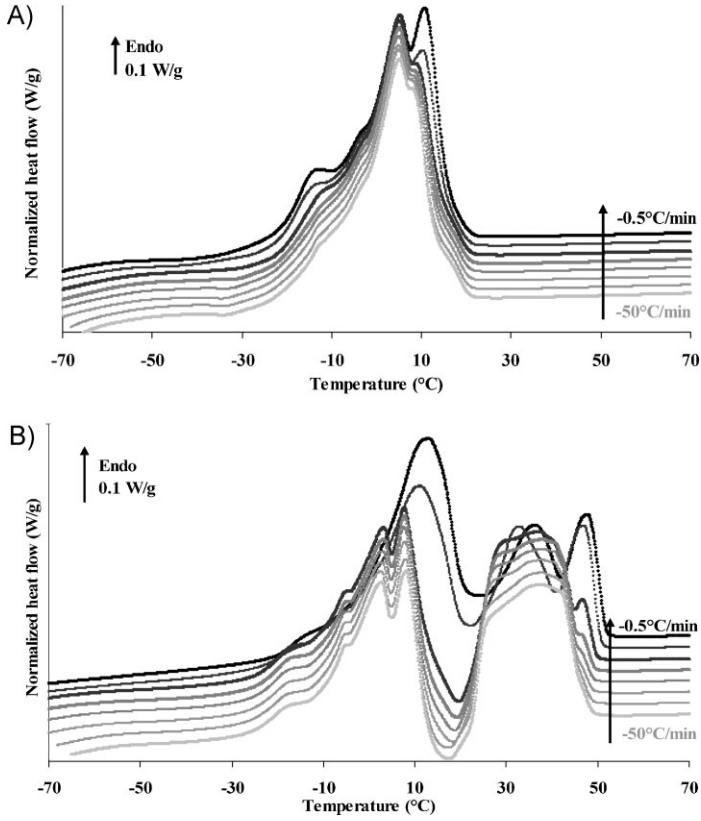

Figure 6.
Differential scanning calorimetry melting curves for liquid fraction (A) and solid fraction (B) obtained by centrifugation of palm oil at different cooling rate of $-0.5\,^{\circ}$C/min to $-50\,^{\circ}$C/min. The signals are quasi identical for (A), while a threshold for a cooling rate of $-5\,^{\circ}$C/min is observed for (B).

crystal size and referred to a triclinic parallel subcell. The β' form less stable referred to an orthorhombic perpendicular subcell. And the α form is the least stable form with a bigger crystal size referred to an hexagonal subcell. The melting behaviour of crystalline form of fats is determined by the polymorph presents.[14] Usually, the melting points of the β' and the β polymorphs of TAGs are higher than for the α polymorph of the same TAGs. Thus, the differences observed in melting are due to the existence of a different polymorphism. We could suppose that a slower cooling rate permits the formation of β' or β form against α form. Nevertheless, we noted in the figure 3 a threshold for a cooling rate of $-3\,^{\circ}$C/min. It seems that TAGs molecules did not succeed in the most stable arrangement in continuation of this cooling rate ($-3\,^{\circ}$C/min). From DSC melting curves we obtained the solid fat content (SFC) of palm oil in function of temperature (Figure 7). We observed a higher crystallization rate for a slow cooling rate. A fast cooling rate induced just a partial crystallization (less than 90% was obtained at $-30\,^{\circ}$C).

Results for liquid and solid fractions obtained by centrifugation showed the weak variation of polymorphism due to liquid fraction but the huge effect of the solid fraction. The solid fraction seems to induce the variation of polymorphism when the cooling rate changed. The endotherm with higher melting point in slow cooling rate curves might be associated with the development of the β' or β polymorph against the α polymorph. The growth of these more stable crystalline forms is inducing by the time during the cooling. The time allows to TAGs chains to reorganize them. From DSC melting curves we obtained the solid fat content (SFC) of liquid fraction and solid fraction of palm oil in function of temperature (Figure 7).

For the liquid fraction, curves have the same profile (figure 8 -A-), but for the solid fraction we observed many differences, more precisely in the average of $20\,^{\circ}$C (figure 8 –B-).

The value of the threshold for the solid fraction is for $q = -5\,^{\circ}$C/min, is weakly different to the value obtain for palm oil. For the two fractions; we observed a higher crystallization rate for a slow cooling rate. A fast cooling rate induced just a partial crystallization. The gap of crystallization at $-30\,^{\circ}$C according to the cooling rate is more important for the solid fraction (less than 80% was obtained at $-30\,^{\circ}$C for a fast cooling rate) than for the liquid fraction (an

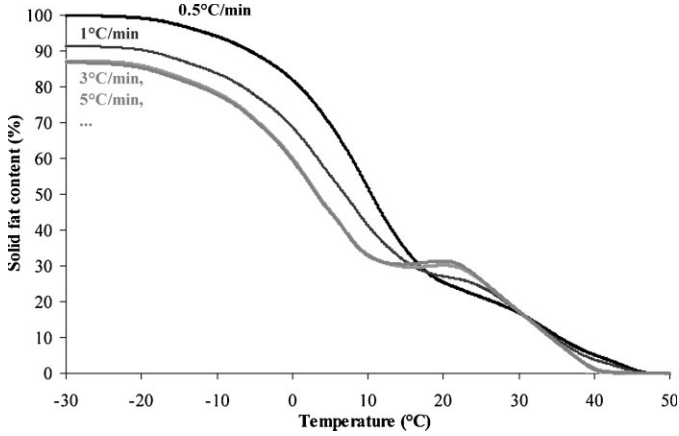

Figure 7.
Evolution of the solid fat content as a function of temperature determined in palm oil at different cooling rates using differential scanning calorimetry. A fast cooling rate does not permit to obtain a totally crystallize product, it stays more than 10% of liquid phase in oil after the cooling at -30 °C.

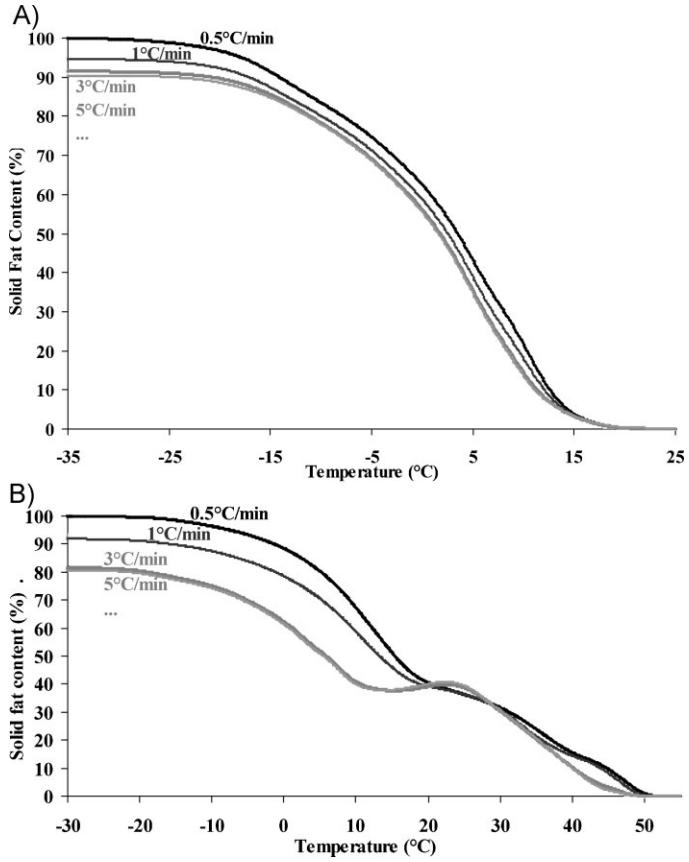

Figure 8.
Evolution of the solid fat content as a function of temperature determined in liquid fraction (A) and solid fraction (B) obtained by centrifugation of palm oil at different cooling rates using differential scanning calorimetry. For the liquid fraction minor variations are observed while for solid fraction important variations of the solid fat content in function of the cooling rate are observed.

average of 90% was obtained at $-30\,^{\circ}C$ for a fast cooling rate).

View to this result we conclude that the variations observed in palm oil melting after different cooling rate is due to the TAGs in the solid fraction; in a world the stearin fraction trapping some liquid TAGs.

View to the size of crystal the texture could be inducing by the solid fraction. The liquid fraction produced small crystal size, below $22\,\mu m$. The size of $22\,\mu m$ is the threshold of crystal size detection for a sensory perception.[5] Only the very slow cooling rate of palm oil ($q < -3\,^{\circ}C/min$) or solid fraction gave bigger size of crystals (figure 5).

The polymorphism of the palm oil was found to be affected by the cooling rate. This result is in agreement with results proposed and obtained about the pork fat for Sventrup et al.[15], the cocoa butter for Perez-Martinez et al.[16] and milk fat for Lopez et al.[17] Nevertheless, ours results showed a threshold for $q = -3\,^{\circ}C/min$, and the solid fraction more affected than the liquid fraction.

Conclusion

At room temperature the palm oil contains an olein fraction and a stearin fraction, so a liquid and solid part. The study of

centrifugation to isolate this two parts, allows some links between crystalline form and liquid TAGs. The different rates of centrifugations have not effect on the separation. The study of the effect of the cooling rate showed, using DSC, the life of a threshold of $-3\,^{\circ}C/min$ in palm oil crystallization. TAGs of palm oil followed a spherolitic crystal growth mechanism in both cooling rates. A slow cooling rate induced bigger crystal size but a fast cooling rate induced smaller crystal size. These variations in palm oil are due to TAGs of solid fraction. This fraction is composed to crystals and blocked liquid TAGs. Furthermore, the cooling rates greatly influence the solid fat content and the type of crystals present in palm oil. Only a very slow cooling rate of palm oil ($q < -3\,^{\circ}C/min$) optimized the solid fat content, gave bigger size of crystal, and more stable form (melting at higher temperature).

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