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Image analysis of palm oil crystallisation as observed by hot stage microscopy

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ABSTRACT

An image processing algorithm previously used to analyse the crystallisation of a pure fat (tripalmitin) has been applied to the crystallisation of a multicomponent natural fat (palm oil). In contrast to tripalmitin, which produced circular crystals with a constant growth rate, palm oil produced speckled crystals caused by the inclusion of entrapped liquid, and growth rates gradually decreased with time. This can be explained by the depletion of crystallisable material in the liquid phase, whereas direct impingement of crystals (the basis of the Avrami equation) was less common. A theoretical analysis combining this depletion with assuming that the growth rate is proportional to the supersaturation of a crystallisable pseudo-component predicted a tanh function variation of radius with time. This was generally able to provide good fits to the growth curves. It was found that growth rate was a relatively mild function of temperature but also varied from crystal to crystal and even between different sides of the same crystal, which may be due to variations in composition within the liquid phase. Nucleation rates were confirmed to vary approximately exponentially with decreasing temperature, resulting in much greater numbers of crystals and a smaller final average crystal size at lower temperatures.

1. Introduction

Palm oil is the world's most exported vegetable oil. It is a key component in products ranging from soaps to margarine and is one of the few vegetable oils with high amounts of saturated fats, hence it is semi-solid at room temperature. It is typically fractionated into higher value products for specific applications in foods [1]. It is important to understand how palm oil crystallises with particular reference to palm oil fractionation and palm oil applications, but also in advancing our understanding of the crystallisation of fat systems in general. Previous studies of RBD palm oil crystallisation have employed a variety of techniques including Differential Scanning Calorimetry (DSC) [2–4], light microscopy [5], X-ray Diffractometry (XRD) [6] or a combination of these methods, to build up information of polymorphic behaviour and crystallisation kinetics.

Palm oil is a complex mixture of triacylglycerols (TAGs) which can be seen to crystallise into two distinct populations when slowly cooled in a DSC [3,5,7], typically one in the range 30–20 °C ("high melting") and the other below 15 °C ("low melting"). In addition to this, palm oil displays multiple polymorphic forms. The polymorphism of palm oil is most reliably determined from XRD experiments, and is now reasonably well understood. Persmark and co-workers [8], conducting XRD experiments, found the existence of three polymorphs, which were named α, β_1' and β_2'. β_1' was found when samples were cooled at a slow rate to 22 °C. β_2' (which from Persmark's description could actually be a sub- α form) was formed from fast cooling to −15 °C (presumably from "low melting" TAGs), and α and β_1' at intermediate conditions. Another study [9] also found that small amounts of β (affecting only a small proportion of TAGs) could co-exist with β', and that both were stable until melted. Some β forms were also found at temperatures below 0 °C, which were presumed to be due to the low melting fractions. Isothermal XRD experiments (of particular relevance to this study) were performed by Chen et al. [10] from 287 to 295 K. They found that the α form formed initially for $T < 20$ °C (reported as 293 K) but transformed to β' over time (of the order of 30 min). A "two stage" process was also evident from viscosity vs time experiments. Further experiments using light microscopy showed what appear to be β' spherulites growing around an α core. As a consequence of this the authors discussed the possibility that XRD data may not always provide a complete picture of the polymorphic state of a sample if a polymorph at the surface is covering over a different polymorph underneath.

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The kinetics of palm oil crystallisation have also been studied by a number of workers. Kawamura [2] performed isothermal DSC experiments in the range 299–303 K from which Avrami fits to solid fat content (SFC) vs time data were made. He then used light microscopy to directly determine growth rates at these temperatures, and used the growth rate information to back-calculate nucleation rates from the Avrami fit constants. Ng and Oh [4] performed an Avrami fit over a wider range of temperatures but without additional growth rate measurements. Induction time data have also been determined from DSC [5] and fitted to expressions for nucleation rate although it is questionable to do this as these represent physically distinct phenomena [11].

Recently, a method of analysing hot stage microscope images of crystallisation has been reported [12]. This automates the process by which crystals can be counted and size measurements made so that data from a series of images containing potentially large numbers of crystals can be relatively easily extracted and analysed. The method has so far been applied to tripalmitoyl-glycerol (also known as tripalmitin) which is a pure TAG. The aim of this paper is to test whether the computer based method can be successfully applied to palm oil, and useful information regarding nucleation and growth extracted that may not be apparent by manual methods.

2. Materials and methods

2.1. Palm oil

Palm oil was obtained from the Malaysian Palm Oil Board and used without further treatment. The composition as determined by HPLC is given in Table 1 using the following method.

2.2. Compositional analysis by HPLC

TAG analysis was performed using an HPLC instrumentation system equipped with a Hewlett Packard HPLC binary pump system (model HP 1100 Series, Waldbronn, Germany), a variable loop injector system, a column oven and an Agilent refractive index detector (Agilent 1100 Series, Waldbronn, Germany). TAGs were separated on two identical Waters Nova-Pak© C18 (Waters Corp., Darmstadt, Germany) columns connected in series with column dimensions of 3.9 mm internal diameter by 300 mm length (each) and preloaded with silica with a particle size of 4 μm. Both columns were maintained at a temperature of 25 °C. The mobile phase used was a mixture of HPLC-grade acetone (Fisher Scientific, Loughborough, UK) and HPLC-grade acetonitrile (Fisher Scientific, Loughborough, UK) in a volume-to-volume (v/v%) ratio of 63.5:36.5 and the flow-rate was fixed at 1 mL/min. The injection volume was set to 10 μL of 5% (w/v) of oil in chloroform. Attenuation was fixed at 500 × 103 RI units and the refractive index detector was maintained at 35 °C. The total runtime for a single injection was 130 min. Identification of TAGs was made based on the retention time of TAG standards and by comparison with the literature. Peak integrations were carried out using the HPLC ChemStation integration software. Integrated peak areas of TAGs and other minor components were normalised based on the total area of all peaks present in the chromatogram. Other minor components such as MAGs and DAGs were not quantified individually and their concentrations were collocated together and their total concentration expressed as 'Others'. HPLC results were expressed in weight percentages (wt%) [13].

Table 1
Composition of RBD palm oil as determined by HPLC (where O=oleic, L=linoleic, M=myristic, P=palmitic and S=stearic acids).

Component	Area %
Others	5.29
OLL	0.39
PLL	2.27
MLP	0.54
OOL	2.00
POL	10.54
PLP	9.35
MPP	0.44
OOO	4.25
POO	23.87
POP	28.26
PPP	4.79
SOO	2.31
POS	4.36
PPS	0.87
SOS	0.48

2.3. Hot stage microscopy

Optical microscopy experiments were carried out using a Linkam THMS600 variable temperature stage (Linkam Instruments, Tadworth, UK) with a Leitz (Diaplan) microscope coupled to a PixeLink PL-A662 digital camera (PixeLink, Ottawa, Canada) and Linksys 32 software data capture system (Linkam Instruments, Tadworth, UK).

The palm oil was first heated to 70 °C, and a small sample was then weighed on to a circular glass slide which was positioned on a hot plate at approximately 70 °C. Another cover slip was then placed concentrically on top of the drop to ensure a uniform thickness of the sample. A photograph was then taken of each slide which was taken at such an angle as to clearly show all the oil on the slide. From this, the area of the palm oil sample was calculated by counting pixels (using image analysis) and using the known area of the glass cover slip as a reference to convert pixels to area. Such an approach allowed photographs taken at an oblique angle to also be used as the angle would affect the view factors of the cover-slip and the oil equally. The area of the oil combined with the mass of oil weighed on to the slide and an assumed density of 922 kg m^{-3} enabled the thickness of the oil sample to be estimated. The sample was subsequently placed in the temperature stage, where it was heated to 70 °C and held for a minimum of 3 min. Thereafter the sample was cooled at a rate of 30 °C min^{-1} to an isothermal holding temperature. Fast cooling was achieved by passing liquid nitrogen through the stage. Images (1280 × 1024 pixels) were collected during the isothermal holding period, observed via a 10 × objective lens and captured automatically every 30–60 s (depending on the temperature and holding period) using Linksys software. The images were scaled by taking an image of a graticule at the same magnification. The temperature was held at the isothermal holding temperature until no further changes were easily discernible. Samples were then heated at 1 °C min^{-1} to ~70 °C to allow melting or polymorphic transformations to be visualised and held for 3 min before once again cooling to another isothermal temperature.

In the case of holding at 35 °C it was found that crystallisation was possible within approximately 4 h, but that only a few crystals would have formed over the entirety of the slide. A number of attempts were made to catch the initial nucleation event but these were unsuccessful due to the very low probability of viewing the relevant area of the slide as the nucleation event occurs (only ~1/100th of the total slide area is visible under the microscope at any one time). Therefore, while the morphologies of these crystals can be reported, the nucleation and growth data are not.