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Isothermal crystallization kinetics of palm oil as influenced by addition of a commercial phytosterol ester mixture

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1. Abstract

In literature there is a good agreement on the health-promoting effects of phytosterols. However addition of phytosterol esters (PEs) to lipid (containing food products) may influence its crystallization behavior. This study investigated the crystallization kinetics of palm oil (PO) after addition of PEs in high concentrations ($\geq 10\%$). The isothermal crystallization of the PE-PO blends was analyzed at a temperature of 20°C and at a supercooling of 18.7°C using differential scanning calorimetry and time-resolved synchrotron X-ray diffraction. At increasing PE concentrations PO crystallization at an isothermal temperature of 20°C started later, was slower and less crystals were formed. Furthermore a delay in polymorphic transition from α to β' was observed. When the blends were isothermally crystallized at a supercooling of 18.7°C only two of these effects remained: the delay in polymorphic transition and the decrease in crystalline content.

2. Introduction

Palm oil (PO) is derived from the mesocarp of the fruit of the oil palm tree (*Elaeis guineensis*). It has surpassed soybean oil to be the most consumed vegetable oil in the world because of its unique

physical properties¹. It is very stable in the β' crystal form which is the most preferred polymorphic form in food products like margarines and shortenings because β' crystals provide a smooth and glossy texture^{1,2}. Because PO is semi-solid at room temperature, it forms the ideal hard stock to be used in trans free food products³. PO contains many different types of TAG, diacylglycerols, free fatty acids and other minor components and it exhibits different polymorphic forms. The polymorphism of a lipid refers to the packing arrangement of the molecules within the crystals. The α polymorphic form has the lowest thermodynamic stability but it has the ability to transform into the more stable β' or β forms⁴. Polymorphism of PO during isothermal crystallization has been extensively studied by several authors^{3,5-8} who reported that the isothermal crystallization mechanism of PO depends on the degree of supercooling. At a high degree of supercooling, PO displays a two-step crystallization with the formation of α crystals in the first step, followed by a transformation into β' crystals and additional formation of β' crystals directly from the melt. At a lower degree of supercooling PO crystallizes directly into the β' polymorph without the prior formation of an α polymorph. The reported value of the cut-off temperature between both mechanisms varies between 22 and 25°C probably because of a difference in experimental conditions on the one hand and a difference in PO composition on the other hand³.

Phytosterols have recently gained much scientific and commercial interest due to the introduction of cholesterol-lowering foods enriched in plant sterols. Phytosterols contain over 250 different compounds including plant sterols and their hydrogenation products, plant stanols⁹. Since the chemical structure of phytosterols is very similar to that of cholesterol, an optimal intake of plant sterols and stanols decreases the intestinal cholesterol absorption and thereby reduces serum LDL cholesterol concentrations by 6-12%¹⁰. Phytosterols are already present in the normal diet for example in vegetable oils, cereal grains and nuts but the levels are too low to provide significant cholesterol-lowering properties¹¹. The most efficient dietary tool to lower serum cholesterol levels are functional foods enriched with plant sterols. A meta-analysis on 124 studies showed that with an intake of phytosterols of 1.5-3 g/day a dose-dependent reduction in the blood levels of LDL-cholesterol of 7-12.5% can be

obtained¹⁰. Among the currently available sterol-based foods, spreads are by far the most common food type, followed by dairy foods and drinks¹². The concentration of phytosterol esters (PEs) that is applied in the fat phase in these products is in the range of 10 to 50% depending on the product type. Evidently, with only 10% PE addition the chance of reaching the advised daily phytosterol intake remains rather low¹³. Food products with rather high PE loading therefore seem very relevant.

In literature there is a good agreement on the health-promoting effects of phytosterols, however addition of PEs to lipid (containing food products), in particular at high amounts, may influence its crystallization behavior and may thus lead to problems occurring during the production process or with the macroscopic properties of the end product. Fundamental research on this topic is very scarce. In a previous study we examined the effects of the addition of a commercial PE mixture on the non-isothermal crystallization behavior of PO and constructed morphology maps that show the different polymorphic forms as a function of temperature and PE concentration during cooling and heating¹⁴. It was shown that the PEs crystallized separately from PO and formed two different ordered structures, namely the higher-melting truly crystalline structure named PE_x which was composed of stearic and palmitic acid esterified PEs and the lower-melting liquid crystalline structure PE_y which mainly contained phytosteryl oleate.

These results enabled research on the impact of adding PEs to PO under industrially more relevant crystallization conditions. More specifically we investigated the influence of PE addition on the ‘equilibrium’ crystallization state of PO in order to evaluate whether PE addition gives desired properties to the end product¹⁵. It was found that PE addition has a major influence on each level of the crystalline structural hierarchy (primary crystallization, polymorphism, network formation and textural properties). Next to the effects related to the final ‘equilibrium’ state, it would be interesting to verify whether PE addition also influences the crystallization kinetics of PO. Diluting PO with PE is expected to delay or slow down the PO crystallization. The extent of this delay and the impact on the rate of polymorphic transitions is however, less predictable. To the best of our knowledge the effect of adding PEs on the isothermal PO crystallization kinetics has not been reported in literature before. More

generally, the effect of addition of many other types of lipids like mono- and diglycerides on the crystallization kinetics of different edible oils and lipids has been studied, but mostly in low concentrations ($< 10\%$) since these minor components can already influence the kinetics at a concentration $< 0.1\%$ ¹⁶.

Given that the concentration range of PEs in the fat phase of most of the existing cholesterol lowering fat rich food products is 10 to 50%, and because of the relevance of higher concentrations, the objective of this study was to investigate the crystallization kinetics of PO after addition of PEs in high concentrations ($\geq 10\%$). Such information is required to assess the potential impact of adding high PE amounts in related food products under processing conditions. A commercially available mixture of PEs containing different types of phytosterols esterified to different types of fatty acids was added to PO in concentrations of 0, 10, 20, 30 and 40%. The isothermal crystallization of the PE-PO blends was analyzed at a temperature of 20°C to examine the effect of PE addition on the two-step crystallization mechanism of PO. In a second set of experiments crystallization was studied at a constant supercooling of 18.7°C in order to eliminate (obvious) effects on the crystallization kinetics related to differences in degree of supercooling of the blends. Differential scanning calorimetry (DSC) was used to get a first view on the isothermal crystallization mechanism and to provide quantitative data on the kinetic behavior by applying the Foubert model. The crystallization mechanism and the polymorphic behavior was examined with time resolved synchrotron X-ray diffraction (XRD) analyses for selected measurements. Furthermore polarized light microscopy (PLM) was used to study the microstructure of the blends.

3. Materials and methods

3.1. Materials

PO and a commercial PE mixture were donated by Unilever (Vlaardingen, The Netherlands). As determined by solid phase extraction using the method described in Panpipat et al. (2013)¹⁷ the commercial PE mixture had a degree of esterification of $97.7 \pm 1.1\%$. The sterol composition of the commercial PE mixture as measured by the method described in Ryckebosch, Bruneel, et al. (2012)¹⁸ is

shown in Table 1. Table 2 depicts the fatty acid composition of PO and the commercial PE mixture as determined by the method described in Ryckebosch, Muylaert, & Foubert (2012)¹⁹. Finally, Table 3 reports the triglyceride (TAG) composition of the PO which was analyzed in a high-performance liquid chromatograph (Waters, Belgium) coupled with a refractive index detector (Waters, Belgium). A NOVA-Pak C18 column (4 μ m, 150 $\times$ 3.9 mm; Waters, Belgium) was used at 30°C. The mobile phase was acetonitrile–acetone (37.5:62.5, vol/vol). PO was solubilized in a chloroform/methanol mixture (1:1, vol/vol) at a concentration of 20 mg/ml and samples of 20 μ l were injected in duplo.

3.2. Blend preparation

PEs were added to PO in concentrations of 10, 20, 30 and 40% by weight. The samples were prepared after complete melting of the lipids at 80°C for 30 min under continuous stirring to obtain a homogeneous, clear mixture.

3.3. Differential Scanning Calorimetry

The DSC experiments were performed using a DSC Q2000 (TA Instruments, Brussels, Belgium) equipped with an autosampler. About 15 mg of melted lipids was sealed into hermetic aluminum pans (TA Instruments, Brussels, Belgium). Three different types of DSC experiments were performed on the pure PO and the PE-PO blends: isothermal crystallization experiments, stop-and-return experiments and determination of the melting curve after reaching the ‘equilibrium’ state to determine T_{offset} . All measurements were carried out at least five times. For the isothermal crystallization experiments the following time-temperature program was used: equilibration at 80°C during 15 min to ensure complete melting and to erase the crystal memory, followed by cooling at 10°C/min to the isothermal temperature (20°C or alternatively a degree of supercooling of 18.7°C) and holding at that temperature until crystallization had finished. The Universal Analysis 2000 software version 4.5A (TA Instruments, New Castle, USA) was used to determine the peak maximum of the α peak ($t_{\alpha \text{ peak}}$) which corresponds to the first peak in the isothermal crystallization curve. The start point and the end point of the β' crystallization, which is the second step in the two-step crystallization of PO, were determined using an objective calculation algorithm described by Foubert et al. (2002)²⁰. Next to the two peaks due to the α

and β' crystallization of PO a third peak occurred in the isothermal crystallization curve of the 40% PE blend at a supercooling of 18.7°C indicating PE crystallization. This peak was however not further analyzed. For indicative purposes the isothermal crystallization experiments at a temperature of 20°C were also performed on the pure PEs, but this DSC crystallization curve was also not further analyzed. Stop-and-return experiments were performed as described in detail in Foubert et al. (2008)²¹ for the quantitative analysis of the α crystallization, which is the first step in the two-step crystallization of PO. In this technique the isothermal crystallization was interrupted after the α crystallization, followed by heating the sample at 20°C/min to obtain the melting curve. The end point of the α crystallization was defined as the start point of the β' crystallization which had been determined with the calculation algorithm of Foubert et al. (2002)²⁰. The melting enthalpy of the α polymorph (ΔH_α) was calculated by integration of the melting curve using a horizontal baseline with a fixed end point at 50°C and the starting point being the intersection of the melting curve with the horizontal baseline. A last type of DSC experiment was designed to determine the offset temperature of melting (T_{offset}) of pure PO and the PE-PO blends. As described by Daels et al (2017)¹⁵ a time-temperature profile of 48h at 10°C was used to bring the samples in the 'equilibrium' (or kinetically stabilized) state. Then the samples were heated at 20°C/min to 80°C to obtain the melting profile. T_{offset} of the peak in the melting curve was determined as the inflection point of the melting peak at the high temperature side of the peak, according to Timms (2003)²². The degree of supercooling of the blends was calculated using this T_{offset} .

3.4. Time-resolved Synchrotron X-ray Diffraction

Pure PO and PO with 20 and 40% of PEs added were analyzed by time-resolved synchrotron X-ray diffraction experiments conducted at DUBBLE, the Dutch-Belgian beamline (BM26) at the European Synchrotron Radiation Facility (ESRF; Grenoble, France). Combined small-angle X-ray scattering (SAXS) and wide-angle X-ray diffraction (WAXD) patterns were recorded using a wavelength, λ , of 1.033 Å. The beamline was equipped with a two-dimensional Pilatus 1 M detector to capture the SAXS

signals and a Pilatus 300 K detector to gather the WAXD data. The scattering angles were calibrated with silver behenate and high-density polyethylene standards.

Approximately 15 mg of melted lipids was sealed into hermetic aluminum pans (TA Instruments, Brussels, Belgium) and loaded onto a temperature-controlled sample stage (Linkam HFS 191, Surry, UK). The following time-temperature program was used: equilibration at 80°C during 15 min to ensure complete melting and to erase the crystal memory, followed by cooling at 10 °C/min to the isothermal crystallization temperature which is at a degree of supercooling of 18.7°C and holding at that temperature until crystallization had finished. During the isothermal crystallization period the SAXS and WAXD patterns were captured simultaneously every 12 s. Each time frame consisted of 11.985 s measuring time and 0.005 s data saving. The 2D SAXS and WAXD data were azimuthally averaged using the program ConeX²³, normalized to the intensity of the incoming beam measured by a photodiode placed downstream from the sample and corrected for the empty holder scattering. The scattering angle 2θ ranged from $0.48 < 2\theta < 7.29^\circ$ for SAXS and $6.63 < 2\theta < 21.9^\circ$ for WAXD. A WAXD background correction was executed by taking the average of about ten data points at about $2\theta = 21.9^\circ$ and subtracting this average from all data points. Thereafter, all patterns were normalized to their integral. Identification of the polymorphs was done on the basis of literature data^{3,6,8,14}.

The WAXD data were also quantitatively analyzed to obtain the fractions of the different polymorphic forms as a function of the isothermal time and this is further explained on the basis of Figure 1. For pure PO and PO with 20% PEs the same method was applied to extract the fractions of the α and β' polymorphs from WAXD. First a reference pattern of the amorphous phase ('amorphous reference'; Fig. 1A) was made by averaging the last ten WAXD patterns during the cooling because no crystallization occurred in that stage. The reference pattern of the α polymorph (' α reference') as shown in Figure 1A was obtained by first selecting the WAXD pattern where the intensity of the α peak was maximal and no peaks of other polymorphic forms were present (named ' α frame' in Figure 1A). The peak maximum of the α peak in the ' α frame' was located at $2\theta = 13.2^\circ$ ($d = 4.22$ Å). Then the 'amorphous reference' was scaled to fit in the ' α frame' (named 'scaled amorphous reference' in Figure

1A, scaling factor = 0.91) and this 'scaled amorphous reference' was subtracted from the ' α frame' to acquire the ' α reference'. As a final step the intensities to the left and right side of the peak in the ' α reference' were set to 0, resulting in the 'cleaned α reference'. The reference pattern of the β polymorph was made analogously as shown in Figure 1B. The ' β frame' contained three peaks at $2\theta = 13.4^\circ$ ($d = 4.41 \text{ \AA}$), $2\theta = 13.9^\circ$ ($d = 4.27 \text{ \AA}$) and $2\theta = 15.0^\circ$ ($d = 3.95 \text{ \AA}$). As can be seen on Figure 1B the scaling factor to fit the 'amorphous reference' in the ' β frame' was smaller than for the ' α frame' and equaled 0.85. Subsequently all WAXD patterns were fit with a linear combination of the 'amorphous reference', the 'cleaned α reference' and the 'cleaned β reference' with the intensities of the amorphous phase and the different polymorphic forms as unknown parameters. The area below each of the constituting curves corresponds to the phase specific mass fractions. Via the Excel Solver the sum of the squared residuals of this fit was minimized. For each pattern the sum of the fractions equaled 1.

For the PE-PO blend with 40% PEs the method to extract the fractions of the different polymorphic forms differed a little because only in these crystallization conditions and at this PE concentration the PEs also crystallized separately from PO, more specifically in the form of PE_x . The formation of the PE_x crystals started after the α crystallization and before the polymorphic transition from α to β crystals. Consequently there were no WAXD patterns in which only the PE_x peaks occurred. Therefore to make the ' PE_x frame' for the PE_x reference (see Figure 1C), a WAXD pattern of pure PEs crystallized in the PE_x form at 27°C was used since this temperature is higher than the melting temperature of PE_y , but lower than the melting temperature of PE_x ¹⁴. The ' PE_x frame' contained nine peaks: a weak peak at $2\theta = 7.81^\circ$ ($d = 7.58 \text{ \AA}$), two strong sharp peaks at $2\theta = 9.54^\circ$ ($d = 6.21 \text{ \AA}$) and $2\theta = 9.86^\circ$ ($d = 6.02 \text{ \AA}$), again three weak peaks at $2\theta = 10.2^\circ$ ($d = 5.81 \text{ \AA}$), $2\theta = 11.7^\circ$ ($d = 5.08 \text{ \AA}$) and $2\theta = 12.4^\circ$ ($d = 4.78 \text{ \AA}$), a strong double peak at $2\theta = 13.9^\circ$ ($d = 4.27 \text{ \AA}$) and $2\theta = 14.1^\circ$ ($d = 4.22 \text{ \AA}$) and one stronger peak at $2\theta = 15.2^\circ$ ($d = 3.91 \text{ \AA}$). The scaling factor of the 'amorphous reference' was 0.90 in this case. Furthermore because of overlapping peaks in the WAXD patterns, the fits of most of the WAXD patterns of PO with 40% PEs did not lead to unique solutions when more than one

polymorphic form had crystallized. Therefore determination of the fractions of the different polymorphic forms was done differently for this sample. First the α fraction at an isothermal time of 10 min was determined via the WAXD data using the above described method since at that time no peaks of other polymorphic forms occurred. Then the α fraction as a function of the isothermal time was calculated from the integral of the α peak in the SAXS region between $2\theta = 3.74$ and $2\theta = 3.84$ and scaling of this (time dependent) integral to the WAXD based α fraction at 10 min. Subsequently all WAXD patterns were fitted again with the α phase fraction fixed to the scaled SAXS based value and with the fractions of the amorphous phase, the β' and PE_x crystal form fractions left as adjustable parameters.

3.5. Polarized light microscopy

Using a polarized light microscope Olympus BX51 (Olympus Optical Co. Ltd., Tokyo, Japan) the microstructure of the samples was studied. A drop of melted sample was transferred onto a pre-heated and covered with a thin covering slide. Using a temperature-controlled Linkam PE120 stage (Linkam Scientific Instruments, Surrey, UK) the samples were heated at 80°C during 15 min to ensure complete melting and to erase the crystal memory, followed by cooling at 10°C/min to the isothermal temperature at a degree of supercooling of 18.7°C and holding at that temperature until crystallization had finished. Images of the microstructures were captured with a digital camera Infinity 2 (Lumenera corporation, Ontario, Canada) and the Infinity capture software (Lumenera Corporation, Ottawa, Canada) every minute during the isothermal crystallization.

3.6. Statistical analysis

The obtained mean values of the crystallization parameters were statistically analyzed by one-way ANOVA with SigmaPlot version 11.0 (Systat Software, Drunen, The Netherlands). Furthermore with the same software Tukey tests were applied to determine significant differences between the mean values at a significance level of 0.05.

4. Results and discussion

4.1. Isothermal crystallization at 20°C

4.1.1. Differential scanning calorimetry: qualitative analysis

For each PE concentration (0, 10, 20, 30, 40 and 100%) Figure 2A shows an overlay of one representative DSC isothermal crystallization curve at 20°C. The isothermal crystallization curve of the **pure PO** showed two peaks which according to the WAXD data discussed below (see section 4.2.3) correspond to the crystallization of PO in the α polymorph (indicated as Peak I) and the subsequent polymorphic transition to the β' form followed by additional crystallization of β' crystals directly from the melt (indicated as Peak II). Many other studies reported this two-step crystallization mechanism when PO was isothermally crystallized at 20°C^{3,5-8}. Peak I started immediately after cooling and was therefore not fully separated from the cooling segment in the DSC curve. However, Peak I was fully separated from Peak II. The isothermal crystallization curve of the **pure PEs** consisted of one single crystallization peak that also started immediately after cooling. Based on the melting temperatures of PE_x (48.9°C) and PE_y (19.9°C) as reported by Daels et al. (2017)¹⁴ it is conceivable that this peak is dominated by the crystallization of PE_x. The DSC curve of the pure PEs is only shown for indicative purposes and, in contrast to the other curves, was not quantitatively analyzed. The isothermal crystallization curves of the **PE-PO blends** showed the same two peaks (Peak I and Peak II) as the curve of the pure PO, although shifted to higher crystallization times with increasing PE concentration. No peaks due to PE crystallization could be distinguished, although PE_x crystallization might be hidden below the PO crystallization events for the blend containing 40% PE. Indeed, in the quantitative DSC analysis an unexpected increase of the PO β' share was detected (see section 4.1.2), which might be due to non-legitimately allocated exothermic heat from the PE_x crystallization. Relying on the morphology maps of PO/PE blends reported by Daels et al. (2017)¹⁴ PE is expected to be at least partially in the PE_x crystalline state in the blends of PO with 10-40% PEs at 20°C. However, the same study reported that a large degree of supercooling is required for PE_x to crystallize, which might translate into a large induction time for isothermal crystallization at 20°C. This induction time seems to be longer than the isothermal crystallization time in the present study, except apparently for the sample with 40% PE (see section 4.1.2). Just as in the isothermal crystallization curve of pure PO, Peak I and

Peak II were fully separated in time, irrespective of the PE concentration. For all samples, except for the 10% PEs sample, Peak I was also fully separated from the cooling run (Figure 2B). When looking at the isothermal crystallization curves of the PE-PO blends it can readily be observed that with an increasing PE concentration Peak I and Peak II shifted to longer crystallization times, broadened and became less intense. The lower intensity (i.e. the lower area covered by these peaks) obviously relates to the lower amount of PO present in these blends. However, areas should be calculated quantitatively to determine whether the reduced area simply follows the PO content or whether additional reducing effects are in place (see section 4.1.2 below). For Peak I, the shift and broadening effect can more clearly be seen in Figure 2B.

4.1.2. Differential scanning calorimetry: quantitative analysis

Because the isothermal crystallization curves of pure PO and the PE-PO blends showed the same peaks (Peak I and Peak II), they were quantitatively analyzed in the same way. Since **Peak I** was not fully separated from the cooling segment in the isothermal crystallization curves of pure PO and some of the PE-PO blends it could not be integrated and analyzed in terms of a crystallization model. Therefore Peak I was quantitatively analyzed by determining the time at its peak maximum in the isothermal crystallization curve (t_α) and the melting enthalpy (ΔH_α) obtained by integration of the endothermic melting peak obtained during heating in the stop-and-return experiments in which the isothermal crystallization was interrupted after the α crystallization. Both parameters are shown in Table 4. It was observed that t_α increased strongly as a function of the PE concentration (significant from 20% PE) which indicates the α crystallization was delayed upon PE addition. After addition of 10% PEs to PO t_α was already doubled and when adding 40% PEs t_α increased even more than 20 times. ΔH_α strongly decreased with increasing PE concentration showing that less α crystals were formed when adding an increasing amount of PE. For each PE concentration the decrease in ΔH_α was much larger than expected from the reduced PO amount by adding PE. For example when 40% PEs were added ΔH_α of PO became almost 10 times smaller.

The β' crystallization was analyzed using a well-established model to describe fat crystallization kinetics that was introduced by Foubert et al. (2002)²⁴ and which obeys the following algebraic equation:

$$f(t) = a * \left[1 - \left(1 + (0.99^{1-n} - 1) * e^{(n-1)*K*(t-t_{ind})} \right) \right]^{\frac{1}{1-n}}.$$

When using DSC to follow the crystallization kinetics f is the released crystallization heat [J/g] at time t [h] and increases with time in a sigmoidal way. a is the value for f as t approaches infinity [J/g] which reflects the maximal amount of crystallization and K is the crystallization rate constant [h^{-1}]. t_{ind} [h] is defined as the time needed to reach 1% of the a value. It is a measure for the so-called induction time. n is the reaction order (dimensionless) and is related to the degree of curve asymmetry. A value of 2 for n means the sigmoidal curve is perfectly symmetric²⁴. This model was fitted to the cumulative heat flow curve during the β' crystallization as a function of time. Because the parameters n and K are correlated, n was fixed at 5.2, which was the mean value of n of all measurements after the first fit and the fitting was subsequently redone with n fixed at 5.2. A value of n larger than 2 indicates that the beginning of the crystallization process is faster than the end²⁴. Table 4 gives the parameters resulting from the Foubert model for the different blends. At higher PE fractions, a clearly decreased (significant from 10% PE) indicating that less β' crystals were formed. When adding 30% PEs the decrease in a was larger than the proportional amount of PE added. However, when adding 40% PEs a significantly increased relative to the value for the 30% PE blend. This was probably due to crystallization of PEs in the 40% PE blend as explained above in section 4.1.1. The K value of the blends decreased up to 70% upon increasing the PE concentration, indicating a progressively slowing down β' crystallization. In case of the blend with 40% PEs a small (not significant) increase of K relative to the value obtained for the 30% PE blend was observed which could also be due to PE crystallization in the 40% PE blend. Concerning t_{ind} a strong increase was noticed with increasing PE concentration for all the PE-PO blends demonstrating that the start of the β' crystallization was delayed. In the blend with 40% PEs t_{ind} was the highest being four times larger than t_{ind} of pure PO. This makes sense since in this two-step crystallization, β' crystals are formed by polymorphic transition of α crystals of which the

crystallization also started later upon PE addition. To verify whether the polymorphic transition itself was also effectively delayed, the polymorphic transition time from α to β' ($t_{transition}$) was calculated as the difference between t_{ind} and t_{α} and the values are also represented in Table 4. Clearly $t_{transition}$ increased with an increasing PE concentration (significant from 20% PE) indicating β' crystallization started later not only due to the delayed α crystallization but also because of an increasingly delayed polymorphic transition. After adding 40% PEs $t_{transition}$ more than doubled compared to the value of pure PO. Furthermore also the total melting enthalpy after the isothermal crystallization (ΔH_{tot}) was calculated as the sum of ΔH_{α} and a and is also represented in Table 4. As could be expected from the decreasing trends in ΔH_{α} and a also ΔH_{tot} decreased with an increasing PE concentration (significant from 10% PE). After adding 30% or more PEs ΔH_{tot} of PO was more than halved.

T_{offset} for each of the PE-PO blends was determined from the melting curves of the blends after reaching the 'equilibrium' state and are shown in Table 5. As T_{offset} of the PE-PO blends decreased with increasing PE concentration, the degree of supercooling during the isothermal measurements at 20°C also decreased with increasing PE concentration. Therefore the observed effects as a result of PE addition during isothermal crystallization at 20°C could have been caused by the difference in supercooling rather than being provoked by differences in PE concentration.

4.2. Isothermal crystallization at a constant degree of supercooling of 18.7°C

In order to eliminate the difference in degree of supercooling of the blends, isothermal crystallization was analyzed at a constant supercooling of 18.7°C. The isothermal temperature used for each blend was calculated based on T_{offset} and is shown in Table 5. The DSC data were used to get a first view on the isothermal crystallization mechanism and to provide quantitative data on the kinetic behavior by applying the Foubert model. This time also synchrotron XRD analyses were performed to examine the polymorphic behavior.

4.2.1. Differential scanning calorimetry: qualitative analysis

Figure 3A shows an overlay of representative isothermal crystallization curves of pure PO and PO with addition of different concentrations of PEs at a supercooling of 18.7°C. The curve of pure PO during

isothermal crystallization at 20°C has already been discussed in section 4.1.1. The isothermal crystallization curves of the PE-PO blends with 10 to 30% PEs showed the same two peaks (Peak I and Peak II) as the curve of pure PO and no other peaks (due to PE crystallization) could be visually distinguished. For the blend with 20% PEs it was later confirmed by XRD that PEs did not crystallize during the isothermal crystallization at a supercooling of 18.7°C (see section 4.2.3). The isothermal crystallization curve of the blend with 40% PEs showed one extra peak between Peak I and Peak II which corresponded to the crystallization of PEs (later confirmed by XRD, see section 4.2.3). From Figure 3A and more clearly from the detailed picture of Peak I in Figure 3B it is clear that Peak I was sharp and started immediately after cooling in the isothermal crystallization curves of each of the PE-PO blends. The intensity of Peak I became lower as a function of the PE concentration but there was no noticeable effect of the PE concentration on the width of Peak I. Concerning Peak II the effects of PE addition were clearer than for Peak I. With an increasing PE concentration Peak II became broader, was shifted to later times and its intensity became lower.

4.2.2. Differential scanning calorimetry: quantitative analysis

Since the isothermal crystallization curves at a supercooling of 18.7°C of the PE-PO blends with 10 to 30% PEs showed the same peaks (Peak I and Peak II) as the isothermal crystallization curve of pure PO at 20°C, their quantitative analysis was performed similarly. For the blend with 40% PEs, Peak I was not fully separated from the PE crystallization peak and therefore no stop-and-return analysis could be done on this sample and ΔH_α could not be determined. For the same blend Peak II was fully separated from the crystallization peak of the PEs and was therefore quantitatively analyzed in the same way as Peak II of pure PO during isothermal crystallization at 20°C.

The analyzed parameters for the **α crystallization** (t_α and ΔH_α) of the pure PO and the PE-PO blends during isothermal crystallization at a supercooling of 18.7°C are reported in Table 6. It can be seen that the α crystallization of PO only started later once 40% PEs was added (t_α increased). At lower PE concentrations, no difference was observed with respect to the value of pure PO. The increase of t_α was ten times less compared to when crystallization was executed at 20°C for the sample with 40%

PEs. The observation that the α crystallization of the PE-PO blends during isothermal crystallization at 20°C was strongly delayed as a function of the PE concentration was therefore caused by the difference in the degree of supercooling of the PE-PO blends, since this delay is nearly absent when the isothermal temperature is corrected for this difference in the degree of supercooling. Furthermore, only when at least 20% PEs were added ΔH_α reduced significantly, indicating that besides a less outspoken increase of t_α with increasing PE concentration, also the decrease of ΔH_α is less pronounced at constant degree of supercooling.

Table 6 also gives the parameters of the Foubert model resulting from the fit of the **β' peak** during crystallization at a supercooling of 18.7°C. Less β' crystals were formed with an increasing PE concentration (a decreased, significant from 10% PE). Within experimental error the decrease in a was proportional to the PO amount, except for the sample with 40% PE for which the a value was significantly lower than expected from proportionality. It cannot be excluded that part of the β' signal was to be found in the preceding PE_x crystallization exotherm. The XRD data (see further below) demonstrate that these crystallization events are in fact not well separated in time. Furthermore, according to the XRD data discussed further below it seems that the β' crystallization in the 40% PE sample slowly continues over a large time interval by which definition of the DSC integration base line is rather uncertain. However, it will be demonstrated when discussing the XRD data that indeed the ultimate β' crystalline content is somewhat lower than what can be expected from proportionality. At a concentration exceeding 10% PE, the β' crystallization was also significantly slower compared to pure PO as a function of the PE addition (K decreased). Just as seen in the results for the isothermal crystallization at 20°C in this case addition of 40% PEs did not lead to an increase of K relative to the value obtained for the 30% PE blend. Furthermore β' crystallization started later with an increasing PE concentration as t_{ind} increased significantly for all studied concentrations. In the 40% PE blend t_{ind} was the highest, being almost three times the value of t_{ind} of pure PO. The increase of t_{ind} upon PE addition was smaller compared to that of the isothermal crystallization at 20°C which could be

expected since also the increase of t_{α} was smaller for the PE-PO blends crystallized at a constant supercooling of 18.7°C.

Next to the parameters of the Foubert model, also $t_{transition}$ and ΔH_{tot} of the PE-PO blends crystallized isothermally at a constant supercooling were analyzed. The polymorphic transition started later with an increasing PE addition ($t_{transition}$ increased, significant from 10% PE). The change in $t_{transition}$ is almost equal to that of t_{ind} indicating the delayed β' crystallization is not caused by a delay in α crystallization but is due to a delay of polymorphic transition from α to β' when PEs are added. Finally, ΔH_{tot} decreased significantly compared to pure PO when adding at least 20% PE, but there was no further decrease that exceeded the error estimate if more PEs were added. However, due to the rather large errors involved, the gradual PO dilution and the potential interference of PE crystallization at high PE concentration we refrain from drawing conclusions on potential PE crystallinity differences.

From the DSC analyses it can be concluded that the main effect related to adding PEs that remains after correcting the isothermal temperature of the PE-PO blends for the difference in the degree of supercooling is the delay of polymorphic transition (increase in $t_{transition}$). The fact that this transition occurs at temperatures below the melting temperature of the metastable α form suggests that this is a solid-solid transition rather than a melt-mediated transformation. The addition of PEs to PO in high concentrations (10-40%) probably hinders the solid-state polymorphic transition because the α crystals are physically more isolated from each other. This physical separation requires multiple, nucleation events to trigger the conversion at each separated location. This leads to a globally increased transformation time. A single nucleation event can trigger the conversion over a larger volume when α crystals are well connected, as seems to be the case for pure PO. This hypothesis was later confirmed with PLM (see section 4.4). This effect is probably enhanced by Ostwald ripening, a melt-mediated process whereby the critical size for a stable crystal increases, causing the smaller crystals to dissolve and leading to less and bigger crystals²⁵. Due to Ostwald ripening the isolation of the PO crystals is therefore even more pronounced.

4.3. X-ray diffraction

The different polymorphic forms were unambiguously identified by comparing the SAXS and WAXD patterns that were simultaneously recorded during the isothermal crystallization of pure PO and PO with 20 and 40% PEs at a supercooling of 18.7°C. The mass fractions of the different polymorphs were quantitatively analyzed based on the WAXD patterns. Figure 4 shows the SAXS diffraction patterns of **pure PO** recorded at different moments during the isothermal crystallization and Figure 5A represents the mass fractions of α and β' as function of the isothermal time. Conform the literature the isothermal crystallization of PO at 20°C occurred in two steps^{3,5,6,8}. Immediately after cooling, one strong first order peak at $2\theta = 1.27^\circ$ ($d = 46.7\text{ \AA}$) and one small third order peak at $2\theta = 3.48^\circ$ ($d = 15.4\text{ \AA}$) appeared in the SAXS patterns accompanied by one peak at $2\theta = 13.2^\circ$ ($d = 4.22\text{ \AA}$) in the WAXD patterns. These spacings are characteristic of the α polymorph in a 2L-stacking. The α fraction reached a maximum of about 7% (Fig. 5A). As deduced from the SAXS and WAXD data, the polymorphic transition to β' started after 7 min. From that moment, one strong first order peak at $2\theta = 1.38^\circ$ ($d = 42.9\text{ \AA}$) and a small third order peak at $2\theta = 4.18^\circ$ ($d = 14.1\text{ \AA}$) emerged in the SAXS patterns while the other peaks that were already present started to vanish. From the same moment onwards three peaks could be observed in WAXD at $2\theta = 13.4^\circ$ ($d = 4.41\text{ \AA}$), $2\theta = 13.9^\circ$ ($d = 4.27\text{ \AA}$) and $2\theta = 15.0^\circ$ ($d = 3.95\text{ \AA}$). An isosbestic point occurred in the SAXS patterns between 9 and 14 min since all the patterns of these frames passed through one single point (Fig. 4B). The isosbestic point indicates that the total crystal volume is constant during the polymorphic transition⁸. After 14 min approximately all α crystals were transformed to β' and extra β' crystals were formed directly from the melt. At the end of the isothermal time the mass fraction of β' crystals was circa 15% (Fig. 5A).

The SAXS and WAXD patterns of **PO with 20% PEs** were very similar to those of pure PO since only the typical PO peaks were observed and thus the PEs did not crystallize in this blend (detailed results not shown). Just as for the pure PO, immediately after cooling α peaks appeared in the SAXS and WAXD patterns. Figure 5B shows that the α fraction reached a maximum value of about 7%. It was clear from the SAXS and WAXD patterns that the polymorphic transition started after 9 min and in the

SAXS patterns an isosbestic point was observed from 12 to 24 min after which extra β' crystals were formed directly from the melt. The fraction β' at the end of the isothermal time was approximately 13.5% (Figure 5B).

The isothermal crystallization at 18.7°C supercooling of **PO with 40% PEs** was different from that of pure PO and PO with 20% PEs since the PEs crystallized in this blend. Figure 6 shows the SAXS patterns of PO with 40% PEs recorded at different moments in time during the isothermal crystallization at a supercooling of 18.7°C and Figure 5C represents the mass fractions of the different polymorphic forms that occurred as a function of the isothermal time. Almost immediately after cooling typical α peaks appeared in both the SAXS and WAXD patterns. After 10 min 5 other peaks started to occur in the WAXD patterns at $2\theta = 9.57^\circ$ ($d = 6.23 \text{ \AA}$), $2\theta = 9.80^\circ$ ($d = 6.06 \text{ \AA}$), $2\theta = 10.2^\circ$ ($d = 5.81 \text{ \AA}$), $2\theta = 12.3^\circ$ ($d = 4.81 \text{ \AA}$) and $2\theta = 12.9^\circ$ ($d = 4.61 \text{ \AA}$). These spacings coincided with those of the structured form named PE_x as reported by Daels et al. (2017)¹⁴. Compared to the WAXD patterns, the PE_x peaks in the SAXS patterns at $2\theta = 1.07^\circ$ ($d = 55.5 \text{ \AA}$) (first order) and $2\theta = 2.14^\circ$ ($d = 46.7 \text{ \AA}$) (second order) were broader and had a much smaller intensity and therefore could only be clearly distinguished from about 16 min. Since in the SAXS and WAXD patterns of PO with 40% PEs only peaks with the same spacings as the pure PO and the pure PEs occurred, it can be concluded that the PEs and PO crystallized separately from each other and that no new (compound) crystal structures were formed in this blend. Of the two ordered PE structures described by Daels et al. (2017)¹⁴ the PE_x structure was thus formed during isothermal crystallization of PO with 40% PEs at 18.7°C supercooling, which corresponds to a temperature of 16.3°C. This could be expected from the morphology map of heating reported by Daels et al. (2017) because 16.3°C is higher than the melting temperature of PE_y and lower than the melting temperature of PE_x in the 40% PE blend. Furthermore it was confirmed that the extra peak between Peak I and Peak II in the DSC curve of PO with 40% PEs was correctly assigned to the crystallization of the PEs (see section 4.2.1.) since the polymorphic transition of α to β' only started after PE crystallization had begun, more specifically at 19 min. However the XRD data show that the PE crystallization was not completely finished before the α to β'

conversion started, which demonstrates that these crystallization events were actually not well separated in time in the DSC crystallization curve. Just as for pure PO and PO with 20% PE, an isosbestic point appeared in the SAXS patterns during the polymorphic transition from 20 to 45 min after which extra β' crystals were formed from the liquid phase. The occurrence of an isosbestic point in this time window indicates that the PE_x crystals do not contribute to any scattering in that angular range but that otherwise – just like for pure PO – all β' crystals are generated from existing α crystals in the initial stage. At the end of the isothermal crystallization the blend consisted of 3% PE_x and 7.5% β' crystals.

When the results of the XRD analyses are compared with those of the DSC experiments, very similar trends are observed. Both DSC and XRD results showed that the start of the α crystallization occurred immediately after cooling and was almost not delayed as a function of the PE concentration.

However the decreasing trend in the amount of α crystallization upon PE addition was in the WAXD data only visible when more than 20% PEs was added to PO while ΔH_α of PO (obtained by DSC) already decreased significantly from 20% PEs addition. This difference is probably due to an incomplete crystallization of the samples at the end of the observed crystallization time as shown by the XRD data which resulted in an uncertain definition of the DSC integration baseline.

Concerning β' crystallization t_{ind} as measured by DSC almost equaled the start of the polymorphic transition as observed in SAXS and WAXD, except for the blend with 40% PEs where t_{ind} was higher than expected based on the WAXD data. Also a decreasing trend was observed in the amount of β' crystals formed as measured by DSC (a value of the Foubert model) as well as by XRD, although for the blend with 40% PEs the effect was larger when measured by DSC. These deviations between the DSC and the XRD data for the blend with 40% PE may both be caused by the fact that according to the XRD data the PE crystallization and the α to β' conversion were not well separated in time in the DSC crystallization curve and by the uncertain definition of the DSC integration baseline as explained before.

4.4. Polarized light microscopy

Figure 7 shows the PLM images of the lipid crystal networks of pure PO and PO with 40% PEs during isothermal crystallization at a supercooling of 18.7°C after an isothermal time of 5 min. According to the DSC and XRD results both samples only contained α crystals of PO at that moment. As can be seen in Figure 7A the typical network of spherulites was seen for PO²⁶. The same type of spherulites were also present in the microstructure of the blend with 40% PEs but they were much smaller and also the amount was much smaller. In the blends with 10 to 30% PEs an intermediate microstructure was observed (results not shown). In the microstructure of pure PO the network of α crystals was much denser and more continuous than the microstructure of the blend with 40% PEs which only showed a weak crystal network, but physically isolated α crystals that were positioned far from each other. The increasing lack of a dense crystal network probably delays the solid-state polymorphic transition of α to β' upon an increasing PE addition.

5. Conclusion

After correcting the isothermal temperature of the PE-PO blends for the difference in the degree of supercooling the increasing effect of PE addition on t_{α} had almost completely disappeared compared to the experiments without correction for the difference in the degree of supercooling. The major effect that remained after correcting the isothermal temperature of the PE-PO blends for the difference in the degree of supercooling is the delay of polymorphic transition (increase in t_{ind} and $t_{transition}$). This solid-solid transformation needs to be nucleated as well and when not well connected, each α crystal aggregate needs to nucleate on its own, which delays the global transformation process. When well connected, the transformation can travel through the network without the need for frequent primary nucleation of the solid-solid transformation. As indicated by the PLM images the α crystals were physically more isolated from each other at increasing PE concentrations.

Furthermore it was seen that upon an increasing PE concentration less β' crystals were formed, even after correction for the difference in the degree of supercooling of the blends, although it should be noted that the crystallization of the samples was not fully completed at the end of the observed crystallization time. Of course upon PE addition the amount of PO (forming the β' crystals) that was

present in the blend decreased. Furthermore at low PE concentrations the PEs themselves did not crystallize but acted as a liquid oil, diluting the PO.

From a practical point of view, avoiding the strong delaying effects on the crystallization rate of PE-PO blends could thus be obtained by lowering the crystallization temperature in order to compensate for the degree of supercooling. However the delay of polymorphic transition of α to β' which also increases the necessary crystallization time still remains an issue in that case. However, at larger supercooling, the α crystalline content might have increased to the extent that again a percolating network is formed, which should enhance the solid-solid transformation kinetics. Further research is needed to confirm this hypothesis.

6. Abbreviations

a	maximal amount of crystallization
ΔH_{α}	melting enthalpy of the α polymorph
ΔH_{tot}	total melting enthalpy after the isothermal crystallization
DSC	differential scanning calorimetry
K	crystallization rate constant
PE(s)	phytosterol ester(s)
PLM	polarized light microscopy
PO	palm oil
SAXS	small angle X-ray scattering
$t_{\alpha \text{ peak}}$	peak maximum of the α peak
TAG	triglyceride
t_{ind}	time needed to reach 1% of the a value
T_{offset}	offset temperature of melting
$t_{transition}$	polymorphic transition time from α to β'
WAXD	wide angle X-ray diffraction
XRD	X-ray diffraction

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609 **10. Tables**

610 **Table 1.** Sterol composition (%) of the commercial phytosterol ester mixture. The values show the
611 average of two measurements. The error is 0.2%.

Sterol	
Brassicasterol	1.3
Campesterol	10.0
Stigmasterol	0.6
β-sitosterol	78.5
β-sitostanol	9.7

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614 **Table 2.** Fatty acid composition (%) of palm oil and the commercial phytosterol ester mixture. The
615 values show the average of three measurements. The error is 0.2%.

Fatty acid	Palm oil	Phytosterol ester mixture
C14:0	1.0	-
C16:0	45.4	6.6
C18:0	4.3	3.8
C18:1	39.6	29.8
C18:2	9.8	59.7

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617

Table 3. Triglyceride (TAG) composition (%) of palm oil. The values show the average of two measurements. The error is 0.02%. Abbreviations: L: linoleic acid, O: oleic acid, P: palmitic acid, M: myristic acid, S: stearic acid

TAG	
PLL	2.71
MLP	0.67
OOL	1.87
POL	10.97
PLP	10.54
OOO	3.62
POO	23.14
POP	31.42
PPP	5.15
SOO	2.49
POS	5.64
PPS	1.08
SOS	0.70

Table 4. Peak maximum of crystallization peak I (t_{α}) for isothermal crystallization, melting enthalpy (ΔH_{α}) of the stop-and-return melting profiles, parameters resulting from the Foubert model fit, transition time ($t_{transition}$) and total melting enthalpy after isothermal crystallization (ΔH_{tot}) for the pure PO and the blends containing 10%, 20%, 30% and 40% phytosterol esters at a crystallization temperature of 20°C. Values show mean $\pm$ standard deviation (n = 5)

%PE	t_{α} (min)	ΔH_{α} (J/g)	a (J/g)	t_{ind} (min)	K (1/s)	$t_{transition}$ (min)	ΔH_{tot} (J/g)
0	0.7 $\pm$ 0.1 ^a	6.1 $\pm$ 0.2 ^a	9.4 $\pm$ 0.4 ^a	8.5 $\pm$ 0.2 ^a	10.9 $\pm$ 0.5 ^a	7.8 $\pm$ 0.2 ^a	15.5 $\pm$ 0.6 ^a
10	1.1 $\pm$ 0.2 ^a	4.7 $\pm$ 0.1 ^b	8.0 $\pm$ 0.0 ^b	10.0 $\pm$ 0.2 ^b	9.3 $\pm$ 0.9 ^b	8.9 $\pm$ 0.3 ^a	12.6 $\pm$ 0.2 ^b
20	2.4 $\pm$ 0.7 ^b	2.8 $\pm$ 0.2 ^c	5.7 $\pm$ 0.3 ^c	12.6 $\pm$ 1.1 ^c	5.3 $\pm$ 0.4 ^c	10.2 $\pm$ 1.3 ^b	8.5 $\pm$ 0.5 ^c
30	7.7 $\pm$ 0.6 ^c	1.8 $\pm$ 0.1 ^d	4.5 $\pm$ 0.2 ^d	22.4 $\pm$ 0.7 ^d	3.1 $\pm$ 0.2 ^d	14.7 $\pm$ 1.0 ^c	6.4 $\pm$ 0.2 ^d
40	17.1 $\pm$ 0.9 ^d	0.6 $\pm$ 0.1 ^e	7.1 $\pm$ 0.3 ^e	35.8 $\pm$ 1.2 ^e	3.5 $\pm$ 0.0 ^d	18.7 $\pm$ 1.5 ^d	7.7 $\pm$ 0.3 ^c

Table 5. T_{offset} of the pure PO and the blends containing 10%, 20%, 30% and 40% phytosterol esters (PEs) and the calculated isothermal temperature based on the average T_{offset} inducing a degree of supercooling of 18.7°C for all the blends. T_{offset} shows the average $\pm$ standard deviation (n=5).

% PEs	T_{offset} (°C)	Isothermal temperature (°C)
0	38.7 $\pm$ 0.2 ^a	20.0
10	38.0 $\pm$ 0.3 ^b	19.3
20	36.7 $\pm$ 0.2 ^c	18.1
30	35.9 $\pm$ 0.2 ^d	17.3
40	35.0 $\pm$ 0.4 ^e	16.3

Table 6. Peak maximum of crystallization peak I (t_α) for isothermal crystallization, melting enthalpy (ΔH_α) of the stop-and-return melting profiles, Parameters resulting from the Foubert model fit, transition time ($t_{transition}$) and total melting enthalpy after isothermal crystallization (ΔH_{tot}) for the pure PO and the blends containing 10%, 20%, 30% and 40% PEs at a constant degree of supercooling of 18.7°C. Values show mean $\pm$ standard deviation (n = 5)

%PE	t_α (min)	ΔH_α (J/g)	a (J/g)	t_{ind} (min)	K (1/s)	$t_{transition}$ (min)	ΔH_{tot} (J/g)
0	0.7 $\pm$ 0.1 ^a	6.1 $\pm$ 0.2 ^a	9.4 $\pm$ 0.4 ^a	8.5 $\pm$ 0.2 ^a	10.9 $\pm$ 0.5 ^a	7.8 $\pm$ 0.2 ^a	15.5 $\pm$ 0.6 ^a
10	0.7 $\pm$ 0.1 ^a	5.4 $\pm$ 2.2 ^{a,b}	7.9 $\pm$ 0.3 ^b	10.5 $\pm$ 0.6 ^b	12.1 $\pm$ 1.5 ^a	9.8 $\pm$ 0.6 ^b	13.3 $\pm$ 2.2 ^{a,b}
20	0.7 $\pm$ 0.1 ^a	3.4 $\pm$ 0.5 ^b	7.6 $\pm$ 0.5 ^b	11.7 $\pm$ 0.5 ^c	8.8 $\pm$ 0.8 ^b	11.1 $\pm$ 0.5 ^c	11.1 $\pm$ 0.3 ^b
30	0.8 $\pm$ 0.1 ^{a,b}	3.5 $\pm$ 0.3 ^b	7.8 $\pm$ 0.4 ^b	15.0 $\pm$ 0.8 ^d	7.2 $\pm$ 0.8 ^c	14.2 $\pm$ 0.8 ^d	11.3 $\pm$ 0.6 ^b
40	0.8 $\pm$ 0.1 ^b	-	4.1 $\pm$ 0.1 ^c	23.8 $\pm$ 0.4 ^e	7.1 $\pm$ 0.4 ^c	23 $\pm$ 0.4 ^e	-

Figure 1. Illustration of the method to make the cleaned reference patterns of the different polymorphic forms, being α (A), β' (B) and PE_x , of which a linear combination was used to fit the WAXD patterns of selected samples in order to quantitatively analyze the fractions of the different polymorphic forms as a function of the isothermal time.

Figure 2. Overlay plot of isothermal DSC crystallization curves of the pure palm oil and the pure phytosterol esters (PEs) and the blends containing 10%, 20%, 30% and 40% PEs at 20°C (Exo up) (A) and the same plot but zoomed in on Peak I (B). Peak maxima of Peak I (α crystallization) and Peak II (β' crystallization) are indicated with different symbols above the peak: Peak I (●) and Peak II (○).

Figure 3. Overlay plot of isothermal DSC crystallization curves of the pure palm oil and the blends containing 10%, 20%, 30% and 40% phytosterol esters (PE) at a constant degree of supercooling of 18.7°C (Exo up) (A) and an overlay plot of the same curves, zoomed in on Peak I (α crystallization) (B). Peak maxima of Peak II (β' crystallization) are indicated with a symbol (○) above the peak.

Figure 4. SAXS and WAXD diffraction patterns at different times during the isothermal crystallization of pure palm oil at 20°C.

Figure 5. Mass fraction of the different polymorphs as measured by WAXD during isothermal crystallization at a constant degree of supercooling of 18.7°C. A: pure palm oil (PO), B: PO with 20% phytosterol esters (PEs), C: PO with 40% PEs.

Figure 6. SAXS and WAXD diffraction patterns at different times during the isothermal crystallization of palm oil with 40% PEs at 16.3°C.

Figure 7. Polarized light microscopy images of the lipid crystal networks of pure palm oil (A) and palm oil with 10%, 20%, 30% and 40% PEs (B) during isothermal crystallization at a degree of supercooling of 18.7°C after an isothermal time of 5 min.

TOC Graphic

44x23mm (300 x 300 DPI)

A

B

0% PEs

10% PEs

20% PEs

30% PEs

40% PEs