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The effect of adding a commercial phytosterol ester mixture on the phase behavior of palm oil

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Abbreviations

DSC	differential scanning calorimetry
FA	fatty acid
HMF	high-melting fraction
LMF	low-melting fraction
PE	phytosterol ester
PO	palm oil
SAXS	small angle X-ray scattering
TAG	triglyceride

T_{offset}	melting offset temperature
T_{onset}	crystallization onset temperature
WAXD	wide angle X-ray diffraction
XRD	X-ray diffraction

Summary

The objective of this study was to investigate in depth the non-isothermal crystallization and melting behavior of binary blends of palm oil (PO) with a commercial, multi-component phytosterol ester (PE) mixture. DSC and time-resolved synchrotron X-ray diffraction (XRD) experiments were conducted on blends with a PE concentration from 0 to 100% at intervals of 10% for DSC and 20% for XRD. Based on XRD, two different ordered structures were identified in pure the PEs. The structure designated as PE_x was truly crystalline and needed a very high degree of supercooling for its nucleation from the melt. The structure designated PE_y formed without supercooling and showed long-range order with multiple reflections at small angles but only one broad reflection at high angle, typical of liquid crystalline samples. Furthermore, PE_y had a high tolerance for molecules of different chemical nature. In the PE-PO blends no other ordered structures were formed other than the ones observed in the pure PEs and PO. The peaks in the DSC runs of the PE-PO blends were linked to transitions of the different polymorphic forms. All structural information of the binary blends as a function of concentration and temperature was collected in morphology maps. The binary blends exhibited eutectic characteristics visualized in the morphology maps with a eutectic point at 40% PEs.

1. Introduction

Phytosterols are triterpenes with a chemical structure similar to cholesterol. As shown in Figure 1 they consist of a four-ring steroid nucleus with a hydroxyl group attached to the C-3 atom of the A-ring and an aliphatic side chain attached to the C-17 atom of the D-ring. Phytosterols include over 250 different plant sterols and stanols and related compounds, but the more abundant ones are β -sitosterol, stigmasterol and campesterol. Plant sterols only vary in their side chain. Possible side chain variations include the presence of a branched methyl or ethyl group on the C-24 atom and the presence and position of a single double bond (Piironen et al., 2000). Plant stanols are the hydrogenation products of the sterols, in which the double bond in the ring is eliminated.

The richest natural sources of phytosterols are vegetable oils followed by cereal grains and nuts. Phytosterols are extracted for use in pharmaceutical, cosmetic and food applications (Fernandes &

Cabral, 2007; Piironen, Lindsay, Miettinen, Toivo, & Lampi, 2000). The two major raw materials used for large scale phytosterol extraction are vegetable oils and tall oil (a by-product of the kraft pulping process) (Helminen et al., 2009; Thompson & Grundy, 2005).

Epidemiologic and experimental investigations have shown that phytosterols may offer protection from the most common cancers in Western societies, but their main beneficial health effect lies in reducing circulating cholesterol concentrations. A meta-analysis of 41 trials showed that a 10% reduction in the blood levels of LDL-cholesterol can be obtained with an optimal intake of phytosterols of 2 g/day, beyond which only a marginal effect is observed (Mensink et al., 2003). Furthermore there are indications that each 1% reduction in LDL-cholesterol corresponds to a 1% reduction in coronary heart disease relative risk, especially for subjects at high cardiovascular risk, like those affected by type 2 diabetes (Marangoni & Poli, 2010). The intake of phytosterols to lower LDL-cholesterol exhibits no side effects, except for children and pregnant women who risk a carotenoid deficiency (Nestel et al., 1999; Sioen et al., 2011). Given the fact that the average daily intake of phytosterols in adults only reaches between 150 and 400 mg/day, phytosterols have to be administered in a higher concentration in order to benefit from their health-promoting properties (Ntanios, 2001). One possible administration method is incorporation of phytosterols into a food matrix.

Enrichment of food products with free phytosterols is, however, difficult from a technological and food quality point of view since they are insoluble in water and poorly soluble in dietary fats while their melting points are rather high (about 140–150°C) (Piironen et al., 2000). Consequently, much research was performed to find approaches to overcome these limitations. Esterification of phytosterols at the C-3 position of the A-ring with a fatty acid (FA) to obtain phytosterol esters (PEs) increases their lipid solubility, lowers their melting point and thus facilitates their incorporation into fat containing foods (Ostlund, 2007). PEs are currently already being used as a food ingredient in modern functional food formulations. Margarines and table spreads are ideal vehicles, although cream cheese, salad dressings, and yogurts are also used as delivery systems (Vaikousi et al., 2007). From an analysis of fat rich cholesterol lowering food products like margarines and spreads on the Belgian market it was deduced that the PE concentration that is applied in the fat phase in these products is in the range of 10 to 50% depending on the product type (e.g. light margarine, margarine with butter or olive oil, etc.).

However, addition of PEs to the fat phase of food products 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 (Rodrigues et al., 2007). Fundamental research on this topic is, however, very much lacking. To our knowledge, the influence of the addition of PEs on

the physical properties of edible fats has only been reported twice in the literature. Vu et al. (2004) showed that self-made PEs added to liquid corn oil caused unwanted PE crystallization above a certain threshold concentration. Rodrigues et al. (2007) investigated how the crystallization behavior of milk fat was changed by blending with a commercial mixture of PEs. They showed that adding PEs to milk fat induced a softer consistency and a lower solid fat content. These authors however, did not report any information on the melting and polymorphic behavior of the pure substrates nor their blends since no differential scanning calorimetry (DSC) or X-ray diffraction (XRD) analyses were performed. Furthermore, PEs were only added in two different concentrations giving only a limited insight into the phase behavior of PE blends with milk fat.

The objective of this study was to investigate in depth the non-isothermal crystallization and melting behavior of a commercial PE mixture in blends with palm oil (PO) based on DSC and time-resolved synchrotron XRD measurements and to map the different polymorphic forms as a function of temperature and PE concentration. Although the concentration range of PEs that is applied in the fat phase of most of the existing cholesterol lowering fat rich food products is 10 to 50%, the full concentration range of 0 to 100% PEs in PO was investigated in this study to obtain insight into the phase behavior of the blends. In this study, PO was used as a 'host' fat for PE addition because recently PO has surpassed soybean oil to be the most consumed vegetable oil in the world (Tan & Nehdi, 2012).

2. Materials and methods

2.1. Materials

PO and a commercial PE mixture were donated by Unilever (Vlaardingen, The Netherlands). The degree of esterification in the commercial PE mixture was $97.7 \pm 1.1\%$ as was confirmed by solid phase extraction performed as described in (Panpipat, Xu, & Guo, 2013). Table 1 reports the sterol composition of the commercial PE mixture as measured by the method described in (Ryckebosch, Bruneel, et al., 2012). Table 2 shows the FA composition of PO and the commercial PE mixture as determined by the method described in Ryckebosch, Muylaert, & Foubert (2012). Table 3 depicts the triglyceride composition of the PO used. It was analyzed in a high-performance liquid chromatograph (HPLC) (Waters, Belgium) on a NOVA-Pak C18 column ($4 \mu\text{m}$, $150 \times 3.9 \text{ mm}$; Waters, Belgium) at 30°C with acetonitrile–acetone (37.5:62.5, vol/vol) as the mobile phase. The HPLC was coupled with a refractive index detector (Waters, Belgium) set at 40°C . PO was solubilized in a solvent mixture (1:1 methanol:chloroform) at a concentration of 20 mg/mL and injected in duplo (20 μL). The fatty acid profile and the TAG profile of PO were in good agreement with other studies (Tan & Nehdi, 2012).

2.2. Blend preparation

Binary blends of PEs and PO were prepared with a PE concentration from 0 to 100% with intervals of 10% for the DSC measurements and 20% for the time-resolved synchrotron XRD analyses. The blends were prepared after complete melting of the pure substrates at 80°C for 30 min under continuous stirring. The blends were visually homogeneous and stable, indicating that no liquid-liquid phase separation occurred.

2.3. Differential scanning calorimetry

The DSC experiments were performed using a DSC Q2000 (TA Instruments, Brussels, Belgium) equipped with an autosampler. About 10-20 mg of melted fat was sealed into a hermetic aluminum pan (TA Instruments, Brussels, Belgium). The following time-temperature program was used: equilibration at 80°C for 15 min to ensure complete melting and to erase the crystal memory, followed by cooling at 5°C/min to -55°C to induce crystallization, subsequently holding at -55°C for 5 min and finally heating at 5°C/min to 80°C to obtain the melting profile. Each measurement was repeated five times. The crystallization onset temperature (T_{onset}) of different peaks in the crystallization curve was determined by the intersection of the baseline of the crystallization curve with the absolute highest tangent at the high temperature side of the crystallization peak. The melting offset temperature (T_{offset}) of different peaks in the melting curve was determined as the intersection of the baseline in the melting curve with the absolute highest tangent at the high temperature site of the melting peak.

2.4. Time-resolved synchrotron X-ray diffraction

Time-resolved synchrotron XRD experiments were performed at DUBBLE, the Dutch-Belgian beamline (BM26) at the European Synchrotron Radiation Facility (ESRF; Grenoble, France). Small-angle X-ray scattering (SAXS) and wide-angle X-ray diffraction (WAXD) patterns were recorded simultaneously 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.

About 10-20 mg of melted fat was sealed into a hermetic aluminum pan (TA Instruments, Brussels, Belgium) and loaded onto a temperature-controlled sample stage (Linkam HFS 191, Tadworth, UK). The same time-temperature program was used as for the DSC measurements (section 2.3). During the cooling and heating period SAXS and WAXD patterns were collected simultaneously in consecutive 12 s time frames. 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 ConeX (Gommes & Goderis, 2010), 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.

3. Results and Discussion

3.1. Differential scanning calorimetry

Figures 2 and 3 show the DSC cooling and heating runs of pure PO, the pure PEs and their blends. The results of pure PO were very similar to those reported in earlier studies that used the same cooling and heating rates (Man, Haryati, Ghazali, & Asbi, 1999; Ng, 1990; Ng & Oh, 1994). The cooling run of pure PO exhibited two well separated exothermic peaks with the high and low-temperature peak due to the crystallization of a high-melting fraction (HMF) and a low-melting fraction (LMF) respectively. HMF and LMF are sometimes labelled as the stearin (or “hard”) and the olein (or “soft”) fraction since the former is rich in disaturated and trisaturated TAG, while the latter contains more diunsaturated and triunsaturated TAG (Braipson-Danthine & Gibon, 2007; Man et al., 1999; Tan & Man, 2000; Zhou & Hartel, 2006). T_{onset} of HMF and LMF were $19.9 \pm 0.3^\circ\text{C}$ and $7.7 \pm 0.1^\circ\text{C}$ respectively. The bimodal crystallization behavior was also mirrored in the subsequent heating run, exhibiting two endothermic melting peaks with a clear exothermic event in between, suggesting recrystallization or additional crystallization of the HMF during the heating run. Although the high-temperature peak in the cooling run was very sharp, its melting endotherm in the heating run showed a severe amount of broadening over a temperature range of about 25°C . This could be expected since the composition of the melt is changing during the course of melting. The rather narrow high-temperature crystallization peak is a kinetic effect, as a result of nucleation being postponed to lower temperatures. Once triggered, the crystallization proceeds very rapidly, i.e. over a narrowed temperature interval. It is also clear that the enthalpy of the (broad) high-temperature melting peak was much larger compared with the high-temperature crystallization peak. This is likely due to how the HMF changed in nature or magnitude during the exothermic (re)crystallization during heating. The low-temperature endotherm also showed some broadening, as expected. T_{offset} of the HMF was $40.8 \pm 0.4^\circ\text{C}$ while that of the LMF was $16.7 \pm 0.2^\circ\text{C}$ indicating that the degree of supercooling was much higher for the HMF compared to the LMF. Note that the actual T_{offset} for the LMF could be situated at somewhat higher temperatures because this part of the signal is obscured by the overlapping exothermic heat in this temperature range. Be that as it may, this effect would at most reduce the difference in supercooling and not eliminate it.

The DSC cooling run of the pure PEs contained one crystallization peak with an onset of $18.3 \pm 0.0^\circ\text{C}$, while the heating profile contained two melting peaks, a sharper one with a peak maximum at $16.4 \pm 0.1^\circ\text{C}$ and a very broad, plateau-shaped one with a temperature range of about 30°C and an offset temperature of $48.7 \pm 0.2^\circ\text{C}$. Whether or not an exothermic event occurs in between the endothermic signals cannot be determined from the DSC data. The fact that no such signal is clearly seen, does not mean that it is absent. It could be that its magnitude is too small to overcome the endothermic heat with which it might overlap. XRD measurements are required to further explain the thermal behavior of this material. The plateau nature of the high-temperature melting peak reflects the fact that this product is a mixture, just like PO is.

For the PE-PO blends, it can be seen in Figure 2 that the DSC cooling runs of the blends with a low PE concentration ($\leq 40\%$) were very similar to the cooling runs of pure PO. From a PE concentration of 50% the crystallization peaks of the HMF and the LMF of pure PO became less dominant since the PE crystallization peaks became more distinct and modified the shape of the cooling curve. Interpreting the data in terms of two PE crystallization peaks is justified because the XRD data (to be discussed in section 3.2) revealed that the single crystallization peak of the pure PEs in fact contained two overlapping transitions which split over the temperature domain in blends with PO. As the PE concentration increased, T_{onset} of the blends gradually decreased reaching a minimal value of $13.4 \pm 0.2^\circ\text{C}$ at a PE concentration of 60%. From a PE concentration of 70% T_{onset} gradually increased again to the T_{onset} value of the pure PEs. Correspondingly, Figure 3 shows that the DSC heating runs of the blends with a low PE concentration ($\leq 40\%$) were very similar to the heating run of pure PO since the main peaks were those corresponding to the melting of the HMF and LMF of PO with an exothermic peak in between. From a PE concentration of 50% onwards other peaks started to occur and the high-temperature peak was stretched out at the high-temperature side. This plateau-shaped peak became bigger with increasing PE concentration and was most likely due to the melting of PEs (confirmed with XRD in section 3.2). The observed trend in T_{offset} of the PE-PO blends was analogous to that of T_{onset} , however the minimal value of T_{offset} was reached at a PE concentration of 40% and equaled $37.2 \pm 0.4^\circ\text{C}$.

3.2. Time-resolved synchrotron X-ray diffraction

The different polymorphic forms can only be unambiguously identified by XRD. The simultaneously collected SAXS and WAXD diffraction patterns of the pure PO and the pure PEs during cooling and heating are shown in Figure 4 together with the corresponding DSC cooling and heating runs. The XRD patterns of pure PO were similar to those described for non-isothermal crystallization of PO in literature. During cooling, from 20.4°C the SAXS pattern showed one

peak at $2\theta = 1.27^\circ$ ($d = 46.7 \text{ \AA}$) and the WAXD pattern had one peak at $2\theta = 14.0^\circ$ ($d = 4.23 \text{ \AA}$). This short spacing is characteristic for the hexagonal subcell packing of PO in the α -polymorph, while the long spacing corresponds to the first order reflection of a double chain length (2L) structure (Braipson-Danthine & Gibon, 2007; Sato, Ueno, & Yano, 1999). During further cooling, from a temperature of 4.5°C two extra peaks occurred in the SAXS pattern: a strong peak at $2\theta = 1.08^\circ$ ($d = 54.6 \text{ \AA}$) and a weak peak at $2\theta = 2.17^\circ$ ($d = 27.3 \text{ \AA}$). These peaks correspond to the first ($54.6 \text{ \AA}$) and second order ($27.3 \text{ \AA}$) reflections of a triple chain length (3L) structure. At the same temperature the WAXD pattern showed one strong peak at $2\theta = 13.9^\circ$ ($d = 4.26 \text{ \AA}$) coinciding with the short spacing of the α -polymorph and one medium peak at $2\theta = 15.6^\circ$ ($d = 3.81 \text{ \AA}$). These short spacings are characteristic for the orthorhombic subcell packing of PO in a β_2' -polymorph (Braipson-Danthine & Gibon, 2007). In PO, β' exhibits two sub-forms which have a different thermodynamic stability and different melting points and are assigned with a subscript that increases with decreasing stability. β_2' is thus the low-melting and less stable form of β' which is in some studies (Braipson-Danthine & Gibon, 2007) labelled as sub- α despite its orthorhombic subcell packing (Jacobsberg & Ho, 1976; Kawamura, 1980). When comparing these XRD results with the DSC cooling run, the peaks of the $2L\alpha$ polymorph in the XRD patterns coincided with the crystallization peak of the HMF of PO in DSC, while the XRD peaks of the $3L\beta_2'$ polymorph corresponded to the crystallization of the LMF of PO in DSC. Therefore, in the remaining part of this paper these two polymorphic forms of PO are referred to as HMF_ α and LMF_ β_2' . The XRD patterns of pure PO during heating revealed that LMF_ β_2' was fully melted at 5.8°C since the XRD peaks completely disappeared at this temperature. Furthermore from 10.8°C HMF_ α started to recrystallize in a $2L\beta_1'$ polymorphic form since emerging peaks were observed at $2\theta = 1.4^\circ$ ($d = 42.4 \text{ \AA}$) in the SAXS pattern and at $2\theta = 13.4^\circ$ ($d = 4.41 \text{ \AA}$), $2\theta = 13.9^\circ$ ($d = 4.28 \text{ \AA}$) and $2\theta = 14.7^\circ$ ($d = 4.03 \text{ \AA}$) in the WAXD pattern (Braipson-Danthine & Gibon, 2007; Yap, DeMan, & DeMan, 1989). β_1' is the high-melting and more stable β' form and is referred to as HMF_ β_1' in this study (Braipson-Danthine & Gibon, 2007; Jacobsberg & Ho, 1976; Kawamura, 1979). The polymorphic transition from HMF_ α to HMF_ β_1' was also visible as an exothermic signal in DSC and was complete at 20.8°C . The fact that this transition occurred at temperatures *below* the HMF_ α crystallization temperature implies that this is a solid-solid transition rather than a process involving full melting and recrystallization (Sato, Bayés-García, Calvet, Cuevas-Diarte, & Ueno, 2013). Indeed, on thermodynamic grounds, a material cannot melt at temperatures below its crystallization temperature. Literature reported the same transition from HMF_ α to HMF_ β_1' when PO is kept isothermally at a temperature between 10°C and the HMF_ α crystallization

temperature for a longer period of time (Chen, Lai, Ghazali, & Chong, 2002; De Graef, Foubert, Smith, Cain, & Dewettinck, 2007; Ng & Oh, 1994; Verstringe, Danthine, Blecker, Depypere, & Dewettinck, 2013). Upon further heating HMF- β_1' melted and PO was fully liquid above 42.8°C. Some authors also reported a partial transition from β_1' to β in PO during heating based on DSC melting points (Kawamura, 1979) or the occurrence of a very weak WAXD peak at $d = 4.58 \text{ \AA}$ (Braipson-Danthine & Gibon, 2007). In this study no clear β_1' to β transition could be observed in SAXS nor WAXD, maybe because the peak intensity associated with the β phase was below the detection limit. In summary, the XRD data relate well to the DSC heating run of pure PO with the first endotherm corresponding to the melting of LMF- β_2' , the subsequent broad exothermic peak being related to the solid-solid polymorphic transition of HMF- α to HMF- β_1' and ultimately the high-temperature endothermic peak being due to the melting of HMF- β_1' .

The right side of Figure 4 shows the XRD results for the pure PEs. In the SAXS pattern during cooling from 19.4°C two distinct peaks were observed: a weak peak at $2\theta = 2.11^\circ$ ($d = 28.1 \text{ \AA}$) and a very strong peak at $2\theta = 1.60^\circ$ ($d = 37.0 \text{ \AA}$). Figure 5A shows a more detailed SAXS pattern of the pure PE at -54.5°C. A further two weak peaks at $2\theta = 1.05^\circ$ ($d = 56.2 \text{ \AA}$) and $2\theta = 3.21^\circ$ ($d = 18.5 \text{ \AA}$) were present that are not visible in Figure 4 due to the very high intensity of the peak at $37.0 \text{ \AA}$ and the color scale used. In the WAXD pattern of the pure PEs during cooling (Figure 4) one weak broad peak at $2\theta = 11.3^\circ$ ($d = 5.24 \text{ \AA}$) and five weak to medium-strong sharper peaks at $2\theta = 7.75^\circ$ ($d = 7.63 \text{ \AA}$), $2\theta = 9.69^\circ$ ($d = 6.10 \text{ \AA}$), $2\theta = 11.6^\circ$ ($d = 5.10 \text{ \AA}$), $2\theta = 13.9^\circ$ ($d = 4.29 \text{ \AA}$) and $2\theta = 15.2^\circ$ ($d = 3.92 \text{ \AA}$) emerged from 19.4°C. However, the peak at $4.29 \text{ \AA}$ was also less sharp and could be composed of two partially overlapping peaks. The more detailed WAXD pattern of the pure PEs plotted in Figure 5 shows four more peaks at $2\theta = 9.51^\circ$ ($d = 6.23 \text{ \AA}$), $2\theta = 10.3^\circ$ ($d = 5.76 \text{ \AA}$), $2\theta = 12.1^\circ$ ($d = 4.89 \text{ \AA}$) and $2\theta = 12.7^\circ$ ($d = 4.65 \text{ \AA}$) which were very weak and therefore not visible in Figure 4. Unlike most TAG fats, the PEs showed WAXD peaks at unusually low 2θ values which therefore, do not overlap with the WAXD peaks of PO and thus allow for an unambiguous PE identification in blends with PO. During heating the SAXS peak in Figure 4 at $37.0 \text{ \AA}$ disappeared at 19.9°C, while the one at $28.1 \text{ \AA}$ disappeared at 48.9°C which indicated that the SAXS peaks were linked to two different crystal structures with different melting temperatures although during crystallization they occurred at the same temperature. For the identification of the WAXD and SAXS peaks of the pure commercial PE mixture, the TAG terminology cannot be applied since the molecular structure of PEs is too different from the TAG structure. Moreover, as far as we are aware, XRD data on PEs have not been reported before in literature. Therefore, the crystal structures linked to the SAXS peaks at $28.1 \text{ \AA}$ and $37.0 \text{ \AA}$ are

referred to as PE_x and PE_y respectively. It is also clear that the SAXS peak at 28.1 Å in Figure 4 is actually a second order reflection of PE_x with a first order reflection at 56.2 Å as can be seen in the more detailed SAXS pattern in Figure 5. Furthermore, based on the temperature at which the reflections disappeared during heating the single WAXD peak at 5.24 Å can be linked to PE_y , while the other five short spacings correspond to PE_x . When comparing these XRD results with the DSC runs, it is apparent that the single peak in the DSC cooling run coincides with the simultaneous crystallization of PE_x and PE_y , while the low-melting and high-melting peaks in the DSC heating run correspond to the respective melting of PE_y and PE_x . Relying on the melting points of pure PEs reported by Leeson et al. (2002) combined with the fatty acid profile of the PEs (Table 2) our hypothesis is that PE_x contains mainly stearic and palmitic acid esterified to the phytosterols while phytosteryl oleate is the predominant PE in PE_y . Linoleic acid based PE, although being predominantly present, most likely did not crystallize since according to Leeson et al. (2002) pure β -sitosteryl linoleate already has a very low melting temperature (-5°C), falling below that of PE_y . From Figure 4 it could also be determined that the nature of PE_x and PE_y is quite different. The nucleation of PE_y seems to occur rather swiftly since its formation happened without any noticeable supercooling, while PE_x needed a high degree of supercooling to crystallize. Crystallization without supercooling and the presence of only one single broad WAXD reflection suggests that PE_y is liquid crystalline, rather than truly crystalline (Foubert, Dewettinck, Van de Walle, Dijkstra, & Quinn, 2007). It is of interest in future research to alter the cooling rate to verify whether or not the solidification into PE_x and PE_y remain coincident or if one would precede the other. Without such information, discussing the potential influence of PE_x crystallization on PE_y formation (or vice versa) would remain speculative. Their simultaneous formation might be a coincidence, specific to the cooling rate used. Very clearly, the intensity of the PE_x peaks in the SAXS and WAXD patterns increased during melting of PE_y (Figure 4). This was probably caused by a ‘melting’ (or ‘clearing’, if one wishes to adhere to the terminology of liquid crystals) of PE_y and a partial recrystallization into PE_x . Given the high intensity of the PE_y SAXS peak, it seems that quite a lot of material was involved in this phase. A high tolerance for molecules of different chemical nature is to be expected for liquid crystals while this is less so for true crystals. It can be envisaged that a fraction of high-melting stearic and palmitic acid esterified PEs was enclosed in the PE_y liquid crystals during their cooling-induced formation and that these were liberated during melting and added to the PE_x crystals that were already created during the preceding cooling run.

For the PE-PO blends with a PE concentration of 20, 40, 60 and 80% time-resolved SAXS and WAXD diffraction patterns were also simultaneously collected and analyzed. In the patterns of the

PE-PO blends only peaks with the same spacings as in the pure components occurred (although partially dependent on temperature and concentration). Since no new peaks at other spacings occurred in the XRD patterns, it can be concluded that no other (compound) crystal structures were formed in the PE-PO blends. As the plots are merely superpositions of the already discussed SAXS and WAXD patterns, plots of these five PE-PO blends are not reported in this article. Instead, the occurrence of the different polymorphic forms as a function of concentration and temperature is summarized in two morphology maps for cooling and heating in Figure 6 and Figure 7 respectively. Upon cooling the 20% PE blend, first, HMF $_{\alpha}$ started crystallizing at a temperature of 17.3°C, followed by LMF $_{\beta_2'}$ at - 0.6°C and finally PE $_y$ at - 31.5°C. Typical PE $_y$ peaks could be observed in SAXS, although with a low intensity, but not in WAXD likely due to being too weak. No PE $_x$ typical peaks appeared during cooling. During subsequent heating of the 20% PE blend PE $_y$ fully disappeared at - 20.1°C. After PE $_y$ was fully melted, PE $_x$ typical peaks appeared in WAXD, clearly visible from a temperature of - 5.1°C. Then, LMF $_{\beta_2'}$ disappeared at 2.9°C, followed by a polymorphic transition from HMF $_{\alpha}$ to HMF $_{\beta_1'}$ from 10.9°C to 20.9°C. Finally, PE $_x$ completely melted at 33.9°C and HMF $_{\beta_1'}$ at 40.9°C. During heating, no PE $_x$ peaks were detected in SAXS probably due to being too weak. The observations for the 40% PE blend were analogous to those for the 20% PE blend. When cooling the 40% PE blend, HMF $_{\alpha}$ started crystallizing first at 16.2°C, followed by LMF $_{\beta_2'}$ at -0.6°C and finally, PE $_y$ at -16.4°C. No PE $_x$ peaks were observed. During subsequent heating of the 40% PE blend first PE $_y$ disappeared at - 10.7°C. From that temperature, PE $_x$ typical peaks appeared in WAXD. Next, LMF $_{\beta_2'}$ disappeared at - 1.6 °C followed by a conversion from HMF $_{\alpha}$ to HMF $_{\beta_1'}$ from 10.4°C to 20.4°C. Finally, PE $_x$ and HMF $_{\beta_1'}$ melted simultaneously and completely vanished at 38.5°C. For the 20% and 40% PE blends it is clear that PE $_x$ crystals were only formed during heating by recrystallization of the high-melting PE molecules that were liberated from PE $_y$ during its melting. The order of crystal form (dis)appearance changed for the 60% PE blend. When cooling the 60% PE blend first, HMF $_{\alpha}$ started crystallizing at 13.4°C, followed by PE $_x$ at 7.4°C, LMF $_{\beta_2'}$ at - 7.5°C and finally, PE $_y$ at -11.5°C. During subsequent heating PE $_y$ disappeared at - 7.5°C and LMF $_{\beta_2'}$ did at - 5.1°C. From 10.0°C to 20.0°C, HMF $_{\alpha}$ converted into HMF $_{\beta_1'}$ which ultimately disappeared fully at 35.0°C. Finally, PE $_x$ was no longer observed beyond 45.0°C. For the 80% PE blend the order of events changed again. In this case PE $_x$ appeared first during cooling at 18.2°C, followed by HMF $_{\alpha}$ at 7.2°C. Then, PE $_y$ started forming at -0.8°C. LMF $_{\beta_2'}$ was only formed at - 12.8°C. During subsequent heating, LMF $_{\beta_2'}$ disappeared fully at - 5.1°C, followed by PE $_y$ at 2.9°C. HMF $_{\alpha}$ vanished at 19.9°C and at least partially transformed into HMF $_{\beta_1'}$.

HMF β_1 ' melted completely at 30.8°C, i.e. before PE $_x$ just as in the 60% PE blend. PE $_x$ was fully liquid above 47.8°C in the 80% PE blend. Similar to in the pure PE sample, the intensity of the PE $_x$ peaks in the 60% and 80% PE blends increased after melting of PE $_y$ (mostly seen in WAXD) due to a partial recrystallization into PE $_x$. As was hypothesized before, it is most likely that high-melting PE molecules that were enclosed in the PE $_y$ liquid crystals during cooling, were released again during melting and added to the PE $_x$ crystals that were already formed during cooling.

3.3. Modelling of the phase transitions and coupling to the DSC data

The Hildebrand equation was used to describe the liquid-solid boundaries in the morphology maps of cooling (Figure 6) and heating (Figure 7) in order to extrapolate and interpolate between the experimental data points (at intervals of 20% PEs). The Hildebrand equation is based on thermodynamics and is commonly used to rationalize the phase behavior and miscibility of binary mixtures of chemically pure TAGs (Abes, Bouzidi, & Narine, 2007; Boodhoo, Bouzidi, & Narine, 2009; Zhou & Hartel, 2006) and sometimes – theoretically less rigorously - also of binary mixtures of real, multi-component fats (Toro-Vazquez, Briceño-Montelongo, Dibildox-Alvarado, Charó-Alonso, & Reyes-Hernández, 2000; Vereecken, Foubert, Meeussen, Lesaffer, & Dewettinck, 2009). According to the Hildebrand equation the equilibrium liquidus line in a binary system composed of components A and B can be described by one of the following two equations, depending on the concentration being smaller or larger than the eutectic concentration (X_E):

$$\ln X_A = -\frac{\Delta H_A}{R} \left(\frac{1}{T} - \frac{1}{T_A} \right)$$

$$\ln X_B = -\frac{\Delta H_B}{R} \left(\frac{1}{T} - \frac{1}{T_B} \right)$$

with R being the molar gas constant, X_A the mole fraction of A, ΔH_A the molar heat of fusion of component A and T_A the melting point of component A, X_B , ΔH_B , T_B the same parameters but applied to component B and $0 \leq X_A \leq X_E \leq X_B \leq 1$. This equation can only be used provided both components are ideally miscible in the liquid state and, provided they do not cocrystallize into compound crystals. Furthermore, this equation can only explain the trends in melting offsets, because only in these cases can the melt composition be approximated by the overall material composition X_A or X_B (Berghmans, 1994; Hartel & Kaylegian, 2001).

Considering that both blend components in the present case are in fact already mixtures, a treatment in terms of the Hildebrand equation at most represents a convenient format to reveal trends. Finally, the Hildebrand formalism was also used in the present paper to describe the composition dependent evolution of the crystallization onset temperature for HMF α , LMF β'_2

and PE_x , ignoring the fact that an out of equilibrium situation exists because of the nucleation kinetics.

ΔH_A and ΔH_B were determined by a least squares procedure in which these parameters were varied together with T_A and T_B to fit the experimental data points most ideally with the Hildebrand equation. Since no molar masses can be assigned to a given (multi-species) component, mass fractions rather than mole fractions were used in this fitting procedure.

Clearly, approaching the experimental melting offset and crystallization onset of the blends with the Hildebrand formalism appears to be quite accurate as illustrated by the dashed lines in Fig. 6 and Fig. 7, despite the boundaries of its use clearly being violated. One thing, however, that can reliably be derived is that these transitions are (melt) composition dependent. The melting point depression with increasing dilution of the melting species as covered by the Hildebrand equation, is a result of the increased melting entropy, ΔS , with increasing dilution. After all, the melting point, T_m , of a substance is given by the ratio $\Delta H/\Delta S$, with ΔH the melting enthalpy (Himawan, Starov, & Stapley, 2006). The remixing of the crystalline species with the surrounding liquid during the melting process leads to an entropy increase, which is larger, the larger the dilution of the crystallizable species is. For that reason ΔS increases with increasing dilution and hence T_m decreases. Other phase transitions at lower temperatures than the melting offset and crystallization onset of the blends which were not simulated with the Hildebrand equation also show the same (melt) composition dependency, except for the melting of HMF_α . Such effects do not play a role if the transition happens within one single phase and remixing is not an issue, such as is the case for a solid-solid transition. The fact that the T_{offset} of HMF_α is composition independent, supports the idea that HMF_α disappears in favor of the creation of $HMF_{\beta_1'}$ via a solid-solid transition.

The results on the morphology maps in Figures 6 and 7 can be transferred to the DSC experiments in Figures 2 and 3. The peak maxima in the DSC crystallization and melting curves corresponding to the most likely polymorphic forms are indicated with different symbols above and below the peaks respectively. For PE concentrations of 0-100% with 20% intervals this was done based on the morphology maps in Figures 6 and 7, while the peak maxima in the DSC crystallization and melting curves of the intermediate concentrations were assigned by interpolation. When focusing on the cooling runs of the PE-PO blends (Fig. 2), the crystallization peak maxima of HMF_α , $LMF_{\beta_2'}$, PE_x and PE_y show the same trend as a function of the PE concentration as the T_{onset} of these polymorphic forms in the morphology map of cooling (Fig. 6). The DSC data show a minimal T_{onset} at a PE concentration of 60%, which is consistent with the morphology maps. PE_x and PE_y peaks are hidden under the $LMF_{\beta_2'}$ crystallization peak in the DSC runs of the mixtures

with a PE concentration below 40% and 30% respectively. In the heating runs of the PE-PO blends (Fig. 3) the melting peak maxima of $\text{HMF}_{\beta_1'}$, $\text{LMF}_{\beta_2'}$, PE_x and PE_y show the same trend as a function of the PE concentration as T_{offset} of these polymorphic forms in the morphology map of heating (Fig. 7). The morphology map of heating showed a minimal T_{offset} at a PE concentration of 40%, analogous to the DSC data. For mixtures with a PE concentration below 40% the PE_x melting peaks were hidden under the $\text{HMF}_{\beta_1'}$ melting peak in the DSC runs and could thus not be distinguished. Also, for the mixture with a PE concentration of 90%, the $\text{HMF}_{\beta_1'}$ peak could not be distinguished. Finally, the melting peak maximum of HMF_{α} was not indicated in Figure 3 because in this temperature range the signal is obscured by the overlapping exothermic peak of the polymorphic transition.

3.4. PE-PO phase diagram

The morphology map of heating (Fig. 7) can be considered as a kinetic phase diagram of the PE-PO blends (Boodhoo et al., 2009; Bouzidi & Narine, 2012; Takeuchi, Ueno, & Sato, 2003). Although the thermal protocol used in this study (constant cooling and heating rates) did not produce equilibrium states, the ensuing kinetic binary phase diagram allows analysis of the solubility of the two substrates and may be extrapolated to describe equilibrium states (Bouzidi & Narine, 2012). It can be seen that up to a concentration of 40% PEs the liquidus line decreased with an increasing PE concentration, while when more than 40% PEs were added the liquidus line increased again. A clear eutectic behavior with a eutectic point at the blend with 40% PE was thus observed. The eutectic behavior of the PE-PO blends indicates that the PEs and PO are immiscible in the solid state (Himawan et al., 2006; Latip et al., 2013; Xu, Zhao, Wang, Peng, & Dong, 2016), most probably as a result of their highly different molecular shape (Foubert et al., 2007). In fact, the absence of compound crystals is a prerequisite for the use of the Hildebrand equation. Rodrigues et al. (2007) also observed eutectic behavior in blends of a highly unsaturated commercial PE mixture with milk fat, but only based on solid fat content and consistency measurements.

4. Conclusion

Based on the time-resolved synchrotron XRD results, two different crystal structures were identified in the pure PEs. The crystal structure designated PE_x needed a very high degree of supercooling to induce nucleation and most likely is composed of stearic and palmitic acid esterified PEs. The nature of the crystal structure designated PE_y was quite different, since PE_y crystallized without supercooling and showed only one single broad WAXD reflection, suggesting

that PE_y is liquid crystalline. PE_y is believed to predominantly contain phytosteryl oleate with a high tolerance for molecules of a different chemical nature, such as stearic and palmitic acid esterified PEs. In the PE-PO blends, the PEs and PO crystallized separately from each other and no new crystal structures were formed other than the typical PE and PO polymorphic forms. Based on the analysis of the XRD measurements of the PE-PO blends, morphology maps were constructed which show the occurrence of the different polymorphic forms as a function of concentration and temperature. The peaks in the DSC runs of the PE-PO blends were also linked to the occurrence of the different polymorphic forms as a function of concentration and temperature. The morphology map of heating (Fig. 7) can be considered as a kinetic phase diagram of the PE-PO blends, representing a typical eutectic behavior with a eutectic point at the blend with 40% PEs. This observation implies that the PEs and PO were immiscible in the solid state, most likely as a result of their highly different molecular shape which does not allow for the formation of compound crystals.

The outcome of this study provides the necessary background to investigate and understand PE loaded palm oil systems in a more practical context. It would for example, be interesting to investigate the influence of PE addition on the crystallization and long term isothermal annealing of palm oil and the related structural properties.

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6. Tables

Table 1. Sterol composition (%) of the commercial phytosterol ester mixture. The values show the 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

Table 2. Fatty acid composition (%) of palm oil and the commercial phytosterol ester mixture.
The 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

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

7. Figures

Figure 1. Chemical structure of β -sitosterol (Soupas et al., 2005)

Figure 2. DSC cooling runs of the blends of palm oil with the commercial phytosterol ester (PE) mixture. PE concentrations (%PE) are reported above each curve at the right-hand side of the figure. Peak maxima of the most likely polymorphic forms are indicated with different symbols above the peak: HMF $_{\alpha}$ (■), LMF $_{\beta 2'}$ (□), PE $_x$ (●) and PE $_y$ (○) based on the morphology map of cooling. Abbreviation HMF represents high-melting fraction and LMF low-melting fraction.

Figure 3. DSC heating runs of the blends of palm oil with the commercial phytosterol ester (PE) mixture. PE concentrations (%PE) are reported above each curve at the right-hand side of the figure. Peak maxima of the most likely polymorphic forms based on the morphology map of heating are indicated with different symbols above the peak: HMF $_{\beta 1'}$ (*), LMF $_{\beta 2'}$ (□), PE $_x$ (●) and PE $_y$ (○). Abbreviation HMF represents high-melting fraction and LMF low-melting fraction.

Figure 4. WAXD, SAXS and DSC data for pure palm oil (PO, left) and the pure commercial phytosterol ester mixture (PE, right) during cooling (upper part) and heating (lower part). The WAXD and SAXS intensities are color coded. The DSC heat flow data are depicted upward for the cooling runs and downward for the heating runs. Different polymorphic forms are indicated on the WAXD and SAXS patterns with arrows and on the DSC runs. Abbreviation HMF represents high-melting fraction and LMF low-melting fraction.

Figure 5. SAXS (A) and WAXD (B) diffraction pattern of the pure commercial phytosterol ester mixture at -54.5°C

Figure 6. Morphology map of cooling of the blends of palm oil with the commercial phytosterol ester (PE) mixture composed with the onset temperatures of crystallization of the different polymorphic forms based on X-ray diffraction data. The dotted lines are obtained from the simulation of the experimental transition lines using the Hildebrand equation. Abbreviation HMF represents high-melting fraction and LMF low-melting fraction.

Fig. 7. Morphology map of heating also representing phase diagram of the blends of palm oil with the commercial phytosterol ester (PE) mixture composed with the offset temperatures of melting of the different polymorphic forms based on X-ray diffraction data. The dotted lines are obtained from the simulation of the experimental transition lines using the Hildebrand equation. Abbreviation HMF represents high-melting fraction and LMF low-melting fraction. The liquidus line of the phase diagram is composed with the dotted lines.

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Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Graphical abstract

Highlights:

- Pure commercial phytosterol ester mixture shows two different ordered structures
- Peaks in DSC curves linked to different polymorphic forms
- Morphology maps summarize structural information
- Binary system shows eutectic characteristics

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