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Crystallisation behaviour of palm oil nanoemulsions carrying vitamin E

DSC and synchrotron X-ray scattering studies

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Abstract Four emulsions were prepared using high pressure homogenisation at 300 or 1200 bar and aqueous phases containing 4.5 wt% whey proteins in mixture with 20 wt% palm oil (PO), alone (E300 and E1200), or in which 20 wt% PO was replaced by vitamin (EV300, EV1200). Thermal behaviour of bulk fat and emulsions in the absence or presence of vitamin, as monitored by differential scanning calorimetry (DSC), indicated that fat crystallisation in supercooled melt was delayed in bulk fat in the presence of vitamin and more delayed in emulsions with lower droplet sizes and containing vitamin. These results were supported by (i) isothermal DSC experiments where exothermic peaks occurred at higher holding times and (ii) dynamic DSC experiments which showed lower melting reactions in emulsions with lower droplet sizes and containing vitamin. Synchrotron X-ray scattering measurements performed simultaneously at small and wide angles on fat samples stored at 4 °C for 12 h showed coexistence of 2*L* and 3*L* longitudinal stacking in bulk fat without vitamin, and only 2*L* organisation of TAGs in the presence of vitamin. Trends in the proportions of α, β', β lateral packing in lipid droplets were also observed to be more affected by the presence of vitamin rather than their size, indicating a higher rate of $\alpha \rightarrow \beta' \rightarrow \beta$ polymorphic transformations in the presence of vitamin. Combining data obtained from DSC and X-Ray signals showed that lipid droplets with lower solid fat content, of which a higher proportion of β polymporphs, were those presenting lower size and lower ability for vitamin protection against chemical degradation, which is of great interest for formulation of lipid nanoparticles as bioactive matrix carriers.

Keywords Palm oil · Protein · Nanoemulsions · DSC · X-ray diffraction · Delivery system · Encapsulation · Antioxidant · Vitamin · SAXS · WAXS

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Introduction

α-tocopherol, the main component of vitamin E has antioxidant activity. It stimulates T cells and increases the immune defence system. Recommended daily intake of α-tocopherol is 22.7 mg day^{-1}. Its deficiency enhances risks for cancer, artherosclerosis and coronary heart diseases. Needs of α-tocopherol is enhanced to 600–800 mg day^{-1} for patients of these diseases [1].

Nowadays, α-tocopherol is widely used in the pharmaceutical, food and cosmetic industries. However, its applications can be limited due to decomposition reactions of its unique structural characteristics, under exposure to environmental factors, such as temperature, oxygen and light [2, 3]. Recent studies focused on encapsulation by lipid droplets of sensitive bioactives, such as vitamins [4–8], capsaicin [9], tributyrin [10], co-Enzyme Q [11] and β carotenoids [12, 13]. Oil-in-water emulsions containing small droplet sizes (20–500 nm) have gained their importance in pharmaceutical and cosmetic industries, as drug delivery systems with enhanced drug bio-availability and solubility [14], and more recently in food applications for their additional long-term stability against coalescence and aggregation of lipid droplets.

Previous studies on simple and complex oil-in-water emulsions [14–22] indicated that their structural properties are affected by several parameters including not only the lipid droplet composition and their related crystallisation and polymorphic behaviours, but also their particle size and nature of adsorbed materials. In addition, recent studies performed on α-tocopherol-loaded food nanoemulsions [4, 6] or more recent ones on pharmaceutical nanoemulsions [7, 8] indicated that food [4–6] or more recent ones on pharmaceutical [7, 8] nanoemulsions indicated that such factors affected the degree to which lipid droplets can protect the vitamin against chemical degradation.

Differential scanning calorimetry (DSC) is widely used to monitor thermal transitions, taking place in bulk fats and emulsions, and particularly for evaluation of changes associated with physicochemical parameters such as lipid droplet size, hydrophobic emulsifiers and fat polymorphisms [14–19, 23]. Fat polymorphism was studied by X-ray scattering alone [23] or in combination with DSC [24] for palm oil (PO) and milk fat crystals [25]. However, a few of previous studies were concerned with evaluation of crystalline fat content and polymorphisms in PO-in-water in regards to both long-term stability of lipid droplets against coalescence and also in regards to their degree of protection of bioactives against chemical degradation, as related to their lipid droplet size. Our recent studies were focused on protection of α-tocopherol, as a model of food lipophilic vitamin with antioxidant activity [4, 5], by caseinate-stabilised milk fat nanoemulsions containing either a high proportion of poly-unsaturated fatty acids and low melting temperature triacylglycerols (LMT TAGs), or a high proportion of saturated fatty acids and high melting temperature triacylglycerols (HMT TAGs). α-tocopherol loaded in lipid droplets containing LMT TAG was shown to be less protected than in HMT TAG lipid droplets. Using simultaneous DSC/X-Ray scattering measurements, we observed that the long-term vitamin protection against degradation by lipid nanoparticles containing HMT TAG presented $2L\alpha \rightarrow 2L\beta'$ polymorphic transitions under cooling and re-heating cycle, contrary to LMT TAG droplets presenting only $2L\alpha$ polymorphs [4, 5].

The aim of this study was to quantify the solid fat content and to identify polymorphic forms in α-tocopherol PO droplets differing only by their particle sizes. PO, the world's second most important vegetable oil (after soybean oil) is a mixed vegetable oil. Due to its low price, high thermal and oxidative stability and plasticity at room temperature, almost 90% of the world PO productions are used in food products. It is composed by more than 25 types of triacylglycerols (TAGs) and mainly by fatty acid components of which 44% P (16:0), 39% O (18:1) and 10% L (18:2), indicating around 50% saturated and 50% unsaturated fatty acids [23]. Likely in our previous studies, we used DSC and synchrotron X-ray diffraction (SR-XRD) for characterisation of thermal behaviour of crystalline fat and identification of fat polymorphisms as affected by the presence of vitamin and also by changes in lipid nanoparticle size values. Production and characterisation of protein-stabilised PO emulsions, used as vitamin matrix carriers, were described and the degree to which vitamin was protected against degradation was discussed in terms of effects of their lipid droplet size and nature of adsorbed materials [6].

Materials and methods

Four nanoemulsions were formulated from a mixture of whey protein concentrate (provided by Armor Protein, France) and refined PO (provided by IOI loders Croklaanoils B.V.1520 AA Wormerveer, Netherland) with or without fat substitution by α-tocopherol (Sigma Aldrich, Switzerland). The whey protein sample contained 80% proteins (of which 75% β-lactoglobulin and 25% α-lactalbumin), 6% fat and 7% lactose. The PO sample was constituted by 30.4% POP; 21.1% POO; 9.6% MOP; 8.7% POL; 6.9% PPP; 5.1% POSt, corresponding to approximately 50% saturated, 40% monosaturated and 10% polyunsaturated fatty acids (Braipson-Danthine and Gibon, personal communication).

Emulsion preparation and characteristics

The emulsions were prepared and characterised for their physico-chemical properties as described elsewhere [6]. In brief, 4.5% whey protein solution at pH 6.5 was mixed at 65 °C with 20% lipid phase composed either by melted PO alone or POV, in which 20 wt% was substituted by vitamin. The mixtures were homogenised at 10,000 rpm for 10 min using a Polytron (PT MR 3000, Kinematics AG, Switzerland) to form coarse pre-emulsions, which were passed through a high pressure homogeniser (Niro homogeniser Niro Soavi S.P.A., Gea, Italy) at 300 or 1200 bar for 12 cycles to get emulsions without (E300 and E1200) or with (EV300 and EV1200) vitamin and different sizes. The outlet temperatures of emulsions prepared at 300 and 1200 bar for 12 cycles were close to 65 and 68 °C, respectively, at which PO was in liquid state. Physico-chemical characteristics of the resulting emulsions, such as volume median diameters (D_{50}) of lipid droplets, protein surface concentration (mg m^{-2}) and initial temperature of crystallisation were determined by using different experimental approaches as described in details elsewhere [21]. Physico-chemical characteristics of PO, POV and E300, E1200, EV300 and EV1200 samples are reported in Table 1. They show that the volume median diameters (D_{50}

Table 1 Physicochemical characteristics of vitamin-loaded palm oil lipid droplets in protein-stabilised emulsions produced at 300 or 1200 bar and DSC parameters observed from bulk fat samples and emulsions

Sample name	D_{50}/nm ±3.7	Protein load/mg m^{-2} ±0.2	%Vitamin ± 1.2%		T_c/°C ±0.2	$\Delta_{crys}H$/J g^{-1} ±1	ΔT/°C ±0.4
			0 days	60 days			
PO	–	–	–	–	20.3	53	24.5
POV	–	–	–	–	17.3	33	25.8
E300	313	6.6	–	–	13.7	13	32.2
EV300	386	7.3	99%	78%	7.9	8	35.3
E1200	199	5.6	–	–	12.3	12	33.6
EV1200	238	6.2	85%	57%	6.4	11	35.8

Droplet size (D_{50}), protein surface load and % vitamin were adapted from Ref. [6], see text

in nm) and protein load values (Γ in g.m^{-2}) of lipid droplets were higher in emulsions prepared at 300 than 1200 bar, and the presence of vitamin was accompanied by increased values of D_{50} and Γ values. On the another hand, it was observed that just after the emulsions preparation, the percentage of vitamin incorporation was close to 99 and 85% for emulsions EV300 and EV1200, respectively, indicating a higher vitamin degradation under more drastic thermo-mechanical treatment. After a long-term storage, the vitamin content decreased from 99 to 78% and from 85 to 57% in emulsions EV300 and EV1200, respectively [7].

Calorimetric parameters

Crystallisation behaviour of bulk fat and emulsions was monitored by DSC in scanning and isothermal mode, as described elsewhere [21]. In brief, we used two different calorimeters. Perkin Elmer DSC-7 (Perkin–Elmer, Norwalk, USA) equipment was used in scanning mode for monitoring fat crystallisation and melting in samples of approx 40 mg, under a cooling step at 10 °C min^{-1} from 60 to −15 °C and a re-heating step at the same scan rate. This thermal history was applied for evaluation of the temperature of initial and final heat flow deviations of the DSC signals corresponding to the initial and end temperatures of fat crystallisation (T_c) and melting (T_m), respectively. Temperature difference between T_m and T_c was used for evaluation of supercooling (ΔT). In other experiments, supercooled melt PO samples in bulk or emulsions were cooled to 4 °C and stored at this temperature for 10 h, and then re-heated to 50 °C for evaluation of total heat of melting [$\Delta_{fus}H$ (4 °C for 10 h] of crystalline fat formed under storage at this condition.

Isothermal DSC experiments were performed using μDSCIII (SETARAM Instrumentation, Caluire, France) after cooling cycles to 10 or 4 °C at 1.5 °C min^{-1}, followed by isothermal steps at 10 or 4 °C for 120 min, re-heating to 60 °C, at 0.5 °C min^{-1} to assess PO crystalline fat content in bulk and emulsions in these experimental conditions. In these experiments, fat samples (100–125 mg of emulsions or 20–30 mg of bulk fat) were hermetically sealed in the sample vessel, and undecane or distilled water in the reference vessel for monitoring either bulk fat or emulsions, respectively. Time of crystallisation in the isothermal mode was determined from the maximum heat flow deviation (t_{max}) under the isothermal cycle, and crystalline fat content formed upon the cooling and holding steps was determined from area under the endothermic peaks observed upon the re-heating cycles, which corresponds to the apparent heat of melting [$\Delta_{fus}H$ (T at 120 min] of crystalline fat. For emulsions, the apparent heat of melting was normalised to the unit mass of fat in the samples, considering that 20 wt% or 16 wt% of the mass samples (fat weight proportions in emulsions) is involved in the DSC thermal behaviour. Solid fat content (% SF) values were deduced from changes in $\Delta_{fus}H$ values, as determined for each of the samples, relative to $\Delta_{fus}H$ value obtained from PO sample in bulk phase.

DSC data were collected and analysed by the DSC machine software and by MS Excel 2003.

Fat polymorphisms

X-ray diffraction experiments were performed on the SWING beamline at SOLEIL synchrotron (Saint-Aubin, France) operated at 15 keV. The scattered intensity was reported as a function of the scattering vector $q = 4\pi\sin\theta/\lambda$, where 2θ is the scattering angle and λ the wavelength of the incident beam ($\lambda = 0.828$ Å). The X-ray scattering intensity was collected by a two-dimensional CCD detector. The sample to detector distance was set to 50 cm to cover the whole q-range of interest, from 0.08 to 1.8 $Å^{-1}$. Calibration of the q-range was carried out with pure β-tristearin for wide angles and silver behenate for small

angles. Intensity values were normalised to account for beam intensity, acquisition time and sample transmission. Each powder-like diffraction pattern, displaying a series of concentric rings, was then integrated circularly to yield the intensity as a function of q.

Melted bulk fat samples (PO and POV) and the four emulsion samples were loaded (about 30 µL) into thin quartz capillaries of 1.5-mm diameter (GLAS W. Muller, Berlin, Germany). Samples were thermostated in a microcalorimeter, Microcalix, designed to allow simultaneous DSC and X-ray diffraction measurements, which was placed in the beam pathway [26]. Filled capillaries stored at 4 °C for 12 h before analysis were introduced into the calorimeter previously cooled to 4 °C to maintain the sample physical state reached during storage. SAXS and WAXS patterns were analysed using Origin (OriginLab Corporation, Northampton, USA) and Peakfit (Jandel Scientific, Erkrath, Germany) softwares. XRD signals were fitted to determine position, maximum intensity and integrated intensity (area) of the Bragg reflections.

Results and discussions

Physico-chemical characteristics of the protein-stabilised emulsions, as reported in a recent publication [6], are shown in Table 1. In this contribution, PO samples, in the absence or presence of vitamin at 4:1 weight ratio, were studied in bulk phase or in protein-stabilised emulsions for their crystalline fat content and polymorphisms. Examples of cooling and re-heating DSC signals from which temperatures (T_c) and enthalpy changes ($\Delta_{crys}H$) of fat crystallisation, and supercooling, ΔT are shown in Fig. 1. Corresponding values obtained from PO samples in bulk and emulsions are also reported in Table 1. PO in bulk phase starts its crystallisation at 20.3 °C, while T_c for emulsions was located at lower temperatures (13.7 or 12.3 °C) for PE 300 ($D_{50} = 313$ nm) and PE 1200 ($D_{50} = 199$ nm), respectively. Presence of vitamin led to a decrease in T_c values by 3.0 °C for bulk fat and by 5.8 °C for EV300 ($D_{50} = 386$ nm) and by 5.9 °C for EV1200 ($D_{50} = 238$ nm). Thus, the presence of vitamin was accompanied by a higher decrease in T_c values in emulsions than in bulk fat, but independently of the trend in droplet size changes from 386 nm for EV300 to 238 nm for EV1200. In our conditions, the decrease in T_c values, in parallel with emulsification and/or presence of vitamin, was accompanied by a decrease in $\Delta_{crys}H$ values, as determined from the area under the exothermic peaks observed upon the cooling cycle. These results indicated that energy release, which was lower for emulsions containing lipid droplets with lower size values, decreased more for the emulsion EV1200 containing vitamin. Previous studies [15, 22, 23, 27–29] indicated that emulsification conditions and ingredients complexity affect fat droplet characteristics such as average diameters and size distributions of lipid droplets, composition surrounding surface layers and also crystalline fat contents and polymorphisms. This study indicated that heat-induced calorimetric parameters of emulsions were more affected by vitamin than droplet size values, in agreement with our previous results [5]. Similarly, trend in ΔT, the degree of super cooling (temperature needed to initiate fat crystallisation in globules) was affected by the presence of vitamin, and it increased more in emulsions with lower size (Table 1). Crystalline fat development in more supercooled emulsions seemed to be more slowed down in emulsions with low size droplets and containing small molecular weight molecules [22–30].

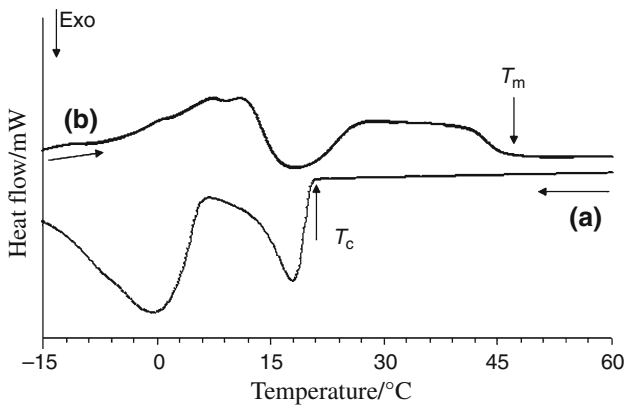

Fig. 1 Example of DSC cooling (**a**) and re-heating (**b**) curves obtained from palm oil at 10 °C min^{-1} scanning rate

Differences in retardation of fat crystallisation in bulk fat and in emulsions differing by their fat droplet sizes were also assessed by isothermal DSC. After cooling from 60 to 4 °C at the highest scan rate allowed by the calorimeter (2 °C min^{-1}), we observed, in addition to the crystallisation peaks observed upon the cooling steps, a sharp peak formed instantaneously at 4 °C, for all of the fat samples. The isothermal DSC study was then conducted at 10 °C after cooling at 1.5 °C min^{-1}, and holding at 10 °C for 2 h. Curves in Fig. 2 observed in these conditions indicated the presence of an instantaneous sharp exothermic peak followed by a much broader exothermic crystallisation peak at different longer time values depending on both bulk or dispersed fat, the lipid droplet size in emulsions and absence or presence of vitamin. The position of the sharp peak was slightly changed, but the broad peak moved from 26 to 40 min for PO and POV bulk fat samples, respectively, and to 47 min for emulsions E300 (313 nm size) and 53 min for E1200 (199 nm size), without vitamin. In the presence of vitamin, any exothermic signal was detected in similar conditions (Table 2).

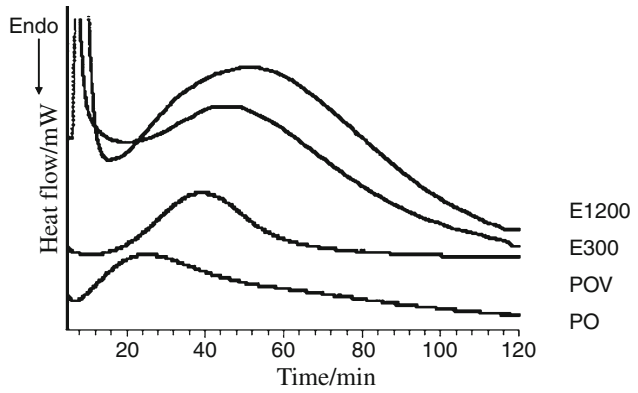

Fig. 2 Isothermal DSC signals observed upon holding at 10 °C for 12 min of bulk palm oil samples in the absence (PO) or presence of vitamins (POV), palm oil emulsions produced at 300 bar (E300) or 1200 bar (E1200) without vitamins

Table 2 DSC parameters observed from bulk and emulsified fat samples. t_{max} is the time corresponding to the broad exothermic peak observed upon 120 min holding time at 10 °C, and % SF values are changes in the solid fat content, relative to palm oil samples in bulk fat, as determined from the melting energy observed upon re-heating the fat samples after storage at 10 °C for 120 min or 4 °C for 600 min (see text)

Fat sample	t_{max}/min ±1.0 min	% SF 10 °C at 120 min ±5%	% SF 4 °C at 600 min ±6%
PO	26	100	100
POV	40	83	74
E300	47	66	68
E1200	53	48	47
EV300	–	46	46
EV1200	–	32	29

Previous studies performed on bulk PO samples [18, 28] showed similar sharp peaks which remained unchanged or vanished, while the broad peak was moved toward longer time with increased temperature of the isothermal cycle. In other studies [27, 29], isothermal crystallisation of tripalmitin solid lipid nanoparticle suspensions at different temperatures was monitored through dynamic DSC signals obtained upon re-heating cycles. Increasing the holding temperature was accompanied by increasingly rapid α to β polymorphic transformations.

Different proportions of crystalline fat contents formed upon holding at 10 °C for 120 min, or upon storage at 4 °C for 600 min, were determined by integration of the endothermic peaks observed upon re-heating the different fat samples. Examples of DSC signals obtained after cooling and holding steps at 10 °C are shown in Fig. 3. The shape of the melting curves of PO samples in bulk phases (PO and POV) showed the presence of low, medium and high

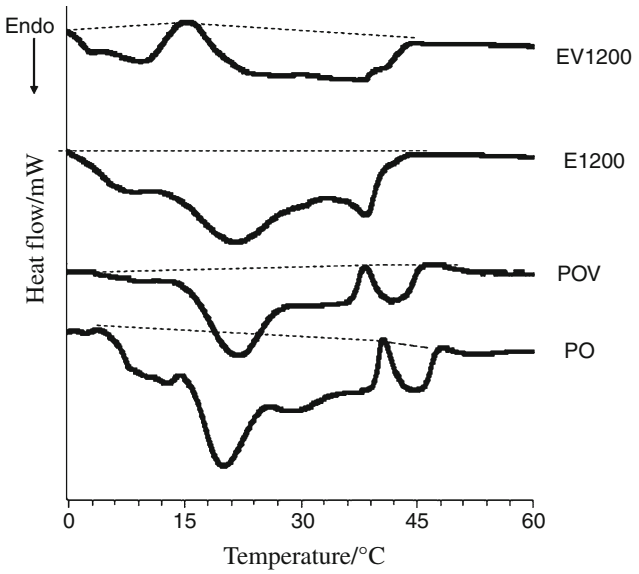

Fig. 3 Dynamic DSC melting curves observed from bulk in the absence (PO) or presence (POV) of vitamins, from emulsions produced at 300 bar (E300), or 1200 bar (E1200) in the absence or presence (EV300, EV1200) of vitamins, after solid fat development during previous isothermal experiments (see Fig. 2)

melting temperature peaks, whereas PO samples in emulsions without vitamin showed only two distinguishable melting peaks and those with vitamin presented one very broad melting peak. % Solid fat content (% SF) was deduced from changes in $\Delta_{fus}H$ values, relative to PO sample in bulk phase and absence of vitamin. Results in Table 2 showed similar values of % SF (10 °C at 120 min) and %SF (4 °C at 600 min) within our experimental uncertainties, which could indicate that the major proportion of crystalline fat content was probably formed during the cooling step. The decrease in the % SF values was higher in the bulk fat sample containing vitamin, and in emulsions with lower size lipid droplets containing vitamin.

Fat polymorphisms

Investigation of crystalline structures formed in bulk PO and emulsions, with or without vitamin E, was investigated by synchrotron XRD from fat samples which were stored at 4 °C for 12 h. Small and wide angles patterns were recorded simultaneously. Small angle scattering (SAXS) yielded information on the longitudinal organisation of TAG while wide angle scattering reflected FA lateral chain packing (polymorphic forms). In brief, the longitudinal organisations of TAG correspond to double-chain length ($2L$) or triple-chain length ($3L$) lamellar structures while the different possibilities of lateral packing of FA chains lead to three major polymorphic forms, which are α, β', β,

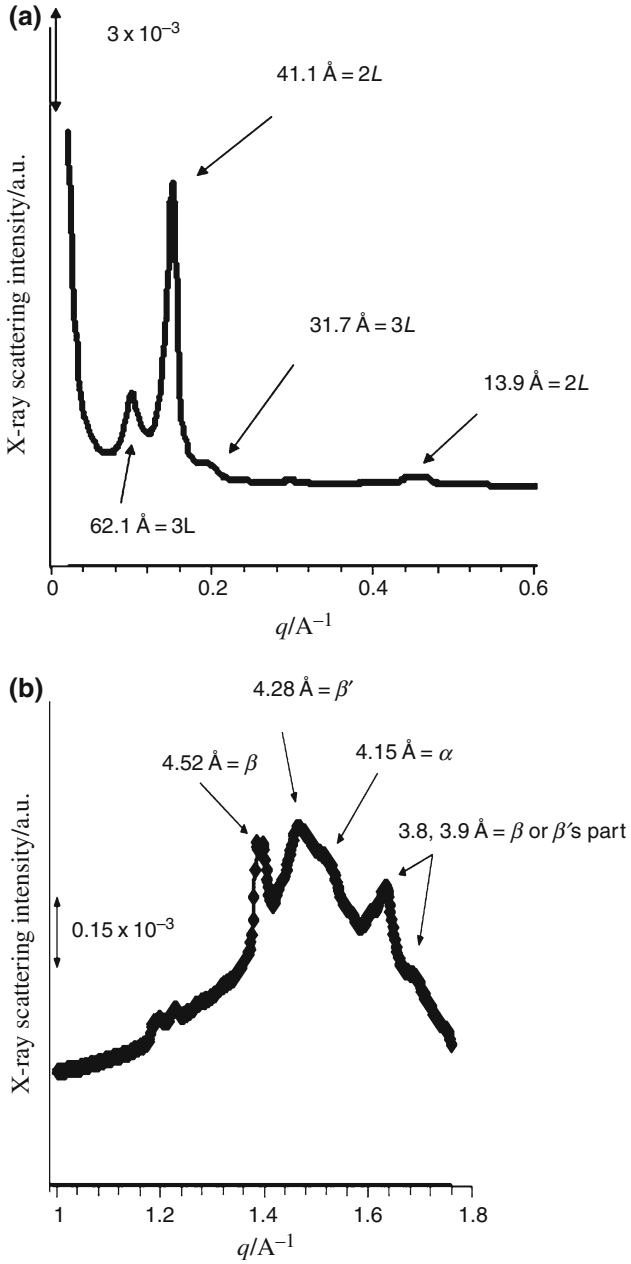

Fig. 4 SAXS patterns (**a**) of palm oil in bulk phase observed at 4 °C, after storage at this temperature for 12 h. WAXS patterns (**b**) of palm oil in bulk phase observed at 4 °C, after storage at this temperature for 12 h

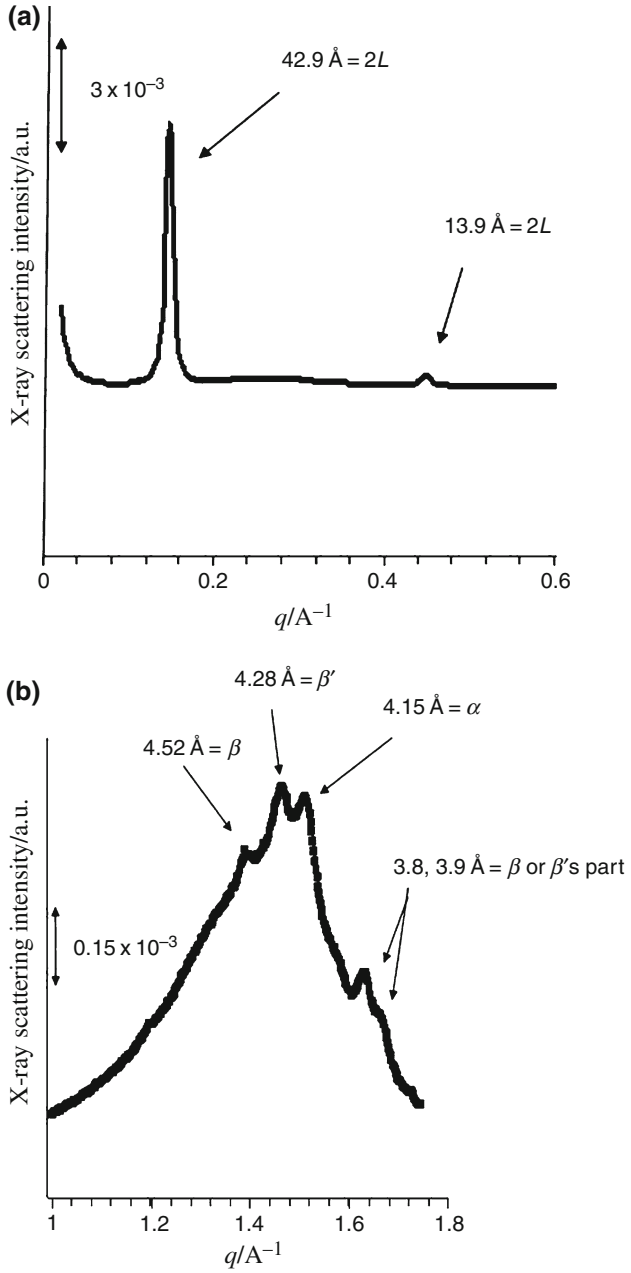

Fig. 5 SAXS patterns (**a**) of palm oil bulk phase in the presence of vitamin observed at 4 °C, after storage at this temperature for 12 h. WAXS patterns (**b**) of palm oil bulk phase in the presence of vitamin observed at 4 °C, after storage at this temperature for 12 h

in increasing order of stability. These subcells correspond, respectively, to a hexagonal (α), orthorhombic (β') and triclinic (β) packing of the acyl chains.

Curves in Fig. 4a and b display SAXS and WAXS signals of bulk PO in the absence of vitamin, and Fig. 5a and b those of PO in the presence of vitamin. In bulk fat, SAXS curve showed five Bragg reflections located at 62.1, 41.1, 31.7, 21 and 13.9 Å. At wide angles, besides two very weak lines at 5.27 and 5.22 Å, diffraction peaks were observed at 4.5, 4.28, 4.15, 3.9 and 3.8 Å. In the presence of vitamin, SAXS pattern revealed only three Bragg reflections at 42.9, 21 and 13.9 Å and the intensity of wide angle peaks decreased. Comparison of SAXS patterns suggests that the peaks at 42.9, 21 and 13.9 Å should be assigned to the first three orders of diffraction of a double-chain length ($2L$) lamellar arrangement while the peaks at 62.1, 31.7 and 13.9 Å should arise from a triple-chain length ($3L$) lamellar structure. Furthermore the results

Table 3 Long spacing of fatty acid chains as observed from X-ray scattering at small angles (SAXS) from the different emulsions which were stored at 4 °C for 12h, and their intensity change relative to palm oil bulk fat sample

Fat sample	Long spacing/Å	% SAXS intensity
E300	42.26	23.1
E1200	42.90	22.7
EV300	41.65	10.1
EV1200	42.90	5.8

reported in Table 3, indicated that peaks of 2*L* lamellar arrangements in lipid droplets of emulsions presented decreasing intensity with droplet size in E300 and E1200, and also with the presence of vitamin.

Regarding the crystalline subcells formed by PO, the peak at 4.5 Å was attributed to a β form, the peak at 4.15 Å could correspond to the α form and the peaks at 4.28 and 3.8 Å could be assigned to a β' form. These tentative assignments were based on the variation of the relative intensities of WAXS lines in emulsions (see below).

In summary, the crystalline structures identified at 4 °C were 2*L* and 3*L* displaying the α, β' and β polymorphic forms in bulk PO and 2*L* associated to the α, β and β' subcells in PO containing vitamin E. Presence of vitamin E increased slightly the α line at 4.15 Å, compared to the β' lines. The different crystal structures could correspond to segregation of TAG species in the solid state, confirming the complex crystallisation behaviour of PO.

The structural behaviour of PO emulsions was significantly different from that of bulk since 3*L* structure was not observed at 4 °C within droplets, whatever their size. However, the dispersion within emulsion droplets led to a decrease in long-spacing difraction intensity (Table 3), they did not impact the nature of crystalline subcells. The coexistence of α, β' and β polymorphic forms was evidenced (Fig. 6).

To better characterise the crystalline structures, the decomposition of the WAXS patterns was performed using PeakFit software. From curves in Fig. 6, where an example of peak decomposition is shown, it appeared that the presence of vitamin, and to a lesser extent the droplet size, affected the relative intensities of the WAXS reflections originating from the different polymorphic forms.

The area associated to each polymorphic form was determined by adding the areas of Bragg reflections (at 4.5, 4.27 and 4.15 Å for β, β' and α, respectively) arising from this crystalline organisation. Noteworthy, the integrated area of all diffraction peaks corresponding to one polymorphic form is proportional to the amount of this form but the proportionality factor may be different depending on the crystal structure. As a result, the proportions of the different polymorphic forms cannot be directly deduced from the relative areas under their Bragg peaks. Nevertheless, the comparison of these areas for the different emulsions provided information on the influence of droplet size and vitamin on the crystallisation behaviour in emulsion. The relative proportions of the α, β' and β subcells (Fig. 7) in crystalline fat content developed upon storage at

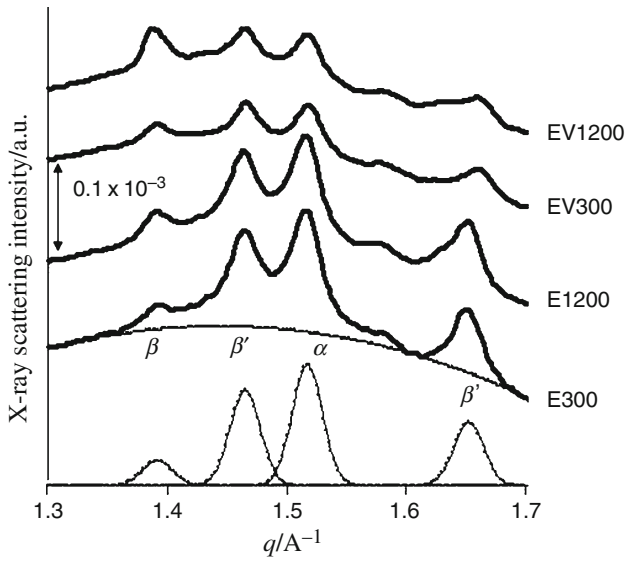

Fig. 6 WAXS patterns observed from palm oil emulsions produced at 300 or 1200 bar in the absence (E300 or E1200 bar, respectively) or presence of vitamins (EV300 or EV1200, respectively) Peaks represented below WAXS signals are deduced from decomposition of WAXS signals (see text)

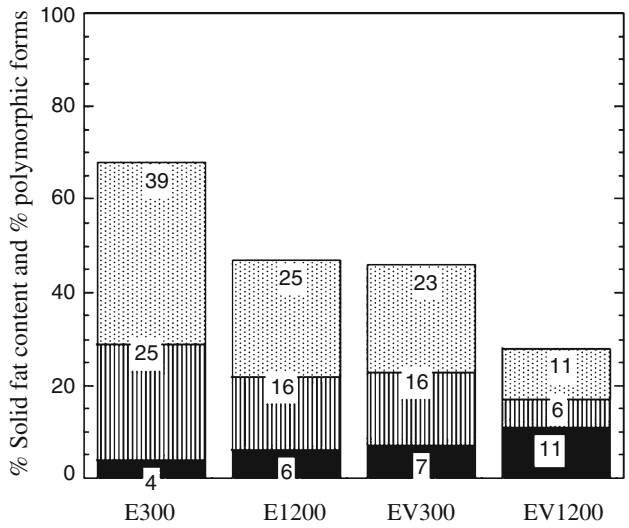

Fig. 7 % solid fat content and its proportion of polymorphic forms in emulsions produced at 300 (E300) or 1200 bar (E1200) in the absence or presence (EV300 and EV1200 bar, respectively) of vitamin. *Top*, *middle* and *bottom* are percentage values of α, β' and β forms, respectively (see text)

4 °C for 600 min (Table 2), expressed from % area of WAXS peaks, are reported in Fig. 7. Regarding the vitamin-enriched PO emulsions (EV300 and EV1200) which differed by their lipid droplet size (386 or 238 nm average size, respectively), the area corresponding to the β form increased at the expense of that related to the α and β' forms. Decreasing the size of the emulsion droplets also led to larger relative area of the β reflection at the expense of both α and β', as shown by comparison of E300 and E1200. The most significant increase in the proportion of the β polymorphic form was evidenced in small emulsion droplets containing vitamin. The relative area of the β reflection increased from about 6% of the total area (or 4% of solid fat content) in E300 emulsion to about 36% (11% *SF*) in EV1200 emulsion, whereas the proportions of both α and β' forms decreased from about 58% (39% SF) to 38% (11% SF) and from 37% (25% SF) to 26% (7% SF), respectively. These changes could correspond to increasingly rapid α to β' or to β polymorphic transformations due to effects of combined lipid droplet size and presence of vitamin.

Thus, combining results reported in Tables 1 and 2, and those in Fig. 7, it appeared that the decrease in the size of lipid particles without vitamin (E300 and E1200) or α-tocopherol-loaded lipid particles (EV300, EV1200) was accompanied upon storage at 4 or 10 °C by a decrease in development of crystalline fat content, of which a higher proportion of polymorphs was in β form.

Conclusions

In this study, we combined dynamic and isothermal DSC and synchrotron X-ray measurements at small and wide scattering angles for evaluation of solid fat content and polymorphisms in PO samples in bulk and dispersed phases, and in the absence or presence of a lipophilic vitamin with antioxidant activity. Extracted data from dynamic and isothermal DSC and X-Ray scattering measurements provided different quantitative parameters, depending on fat samples composition (PO in the absence or presence of lipophilic vitamin), their continuous (bulk) or dispersed physical state (oil-in-water emulsions) containing lipid nanoparticles with different sizes. Comparison between DSC and X-ray peak areas observed from signals corresponding to the different fat samples provided information about crystallisation behaviour of lipid particles in nanoemulsions, and particularly on the influence of vitamin (lipophilic compound), and also lipid droplet size. PO droplets in emulsions with a lower particle size presented a lower solid fat content but a higher proportion of the most stable polymorphic form, β. For vitamin-loaded lipid nanoparticles, the lower solid fat content in EV1200 emulsion could be responsible for a higher mobility of the vitamin within the lipid matrix, whereas the lower particle size values could favor surface exchange reactions between the α-tocopherol-loaded lipid particles and the pro-oxidant aqueous medium. In parallel, our X-ray diffraction study indicated that decreasing the lipid particle size values and the solid fat content was accompanied by a relative increase in the proportion of β polymorphs, which could also contribute to a higher loss in vitamin protection by lipid nanoparticles in EV1200 emulsions than in EV300 emulsions. Our results on structural properties of vitamin-loaded lipid nanoparticles, discussed in terms of their long-term physical stability and protection of lipophilic vitamin upon chemical degradation are of great technological interest for optimisation of bioactive matrix carriers, using generally recognised as safe substances.

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References

1. Sabliov C, Fronczek C, Astete C, Khachaturyan M, Khachatryan L, Leonardi C. Effects of temperature and UV light on degradation of α-tocopherol in free and dissolved form. J Am Oil Chem Soc. 2009;86(9):895–902.
2. Aksu MI. The effect of α-tocopherol, storage time and storage temperature on peroxide value, free fatty acids and pH of kavurma, a cooked meat product. J Muscle Foods. 2007;18(4):370–9.
3. Miquel E, Alegria A, Barbera R, Farre R, Clemente G. Stability of tocopherols in adapted milk-based infant formulas during storage. Int Dairy J. 2004;14(11):1003–11.
4. Relkin P, Yung J-M, Kalnin D, Ollivon M. Structural behaviour of lipid droplets in protein-stabilized nano-emulsions and stability of α-tocopherol. Food Biophys. 2008;3(2):163–8.
5. Relkin P, Jung JM, Ollivon M. Factors affecting vitamin degradation in oil-in-water nano-emulsions. J Therm Anal Calorim. 2009;98(1):13–8.
6. Shukat R, Relkin P. Lipid nanoparticles as vitamin matrix carriers in liquid food systems: on the role of high-pressure homogenisation, droplet size and adsorbed materials. Colloids Surf B Biointerfaces. 2011;86(1):119–24.
7. Hatanaka J, Chikamori H, Sato H, Uchida S, Debari K, Onoue S, Yamada S. Physicochemical and pharmacological characterization of α-tocopherol-loaded nano-emulsion system. Int J Pharm. 2010;396(1–2):188–93.
8. Fangueiro JF, Macedo AS, Jose S, Garcia ML, Souto SB, Souto EB. Thermodynamic behavior of lipid nanoparticles upon delivery of Vitamin E derivatives into the skin: in vitro studies. J Therm Anal Calorim. 2011;103(1):1–8.
9. Choi AJ, Kim CJ, Cho YJ, Hwang JK, Kim CT. Effects of surfactants on the formation and stability of capsaicin-loaded nanoemulsions. Food Sci Biotechnol. 2009;18(5):1161–72.
10. Li Y, Le Maux S, Xiao H, McClements DJ. Emulsion-based delivery systems for tributyrin, a potential colon cancer preventative agent. J Agric Food Chem. 2009;57(19):9243–9.
11. Ozaki A, Muromachi A, Sumi M, Sakai Y, Morishita K, Okamoto T. Emulsification of coenzyme Q10 using gum arabic increases

bioavailability in rats and human and improves food-processing suitability. J Nutr Sci Vitaminol. 2010;56(1):41–7.
12. McClements DJ, Decker EA, Weiss J. Emulsion-based delivery systems for lipophilic bioactive components. J Food Sci. 2007; 72(8):111–23.
13. Yuan Y, Gao Y, Zhao J, Mao L. Characterization and stability evaluation of β-carotene nanoemulsions prepared by high pressure homogenization under various emulsifying conditions. Food Res Int. 2008;41(1):61–8.
14. Müller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of the art. Eur J Pharm Biopharm. 2000;50:161–677.
15. McClements DJ, Dickinson E, Dungan SR, Kinsella JE, Ma JG, Povey MJW. Effect of emulsifier type on the crystallization kinetics of oil-in-water emulsions containing a mixture of solid and liquid droplets. J Colloid Interface Sci. 1993;160(2):293–7.
16. Kaneko N, Horie T, Ueno S, Yano J, Katsuragi T, Sato K. Impurity effects on crystallization rates of *n*-hexadecane in oil-in-water emulsions. J Cryst Growth. 1999;197(1–2):263–70.
17. Awad T, Sato K. Acceleration of crystallisation of palm kernel oil in oil-in-water emulsion by hydrophobic emulsifier additives. Colloids Surf B Biointerfaces. 2002;25(1):45–53.
18. Coupland JN. Crystallization in emulsions. Curr Opin Colloid Interface Sci. 2002;7(5–6):445–50.
19. Relkin P, Sourdet S, Fosseux PY. Fat crystallization in complex food emulsions—effects of adsorbed milk proteins and of a whipping process. J Therm Anal Calorim. 2003;71(1):187–95.
20. Sonoda T, Takata Y, Ueno S, Sato K. DSC and synchrotron-radiation X-ray diffraction studies on crystallization and polymorphic behavior of palm stearin in bulk and oil-in-water emulsion states. J Am Oil Chem Soc. 2004;81(4):365–73.
21. Relkin P, Sourdet S. Factors affecting fat droplet aggregation in whipped frozen protein-stabilized emulsions. Food Hydrocoll. 2005;19(3):503–11.
22. Fredrick E, Foubert I, De Sype JV, Dewettinck K. Influence of monoglycerides on the crystallization behavior of palm oil. Cryst Growth Des. 2008;8(6):1833–9.
23. Braipson Danthine S, Gibon V. Comparative analysis of triacylglycerol composition, melting properties and polymorphic behavior of palm oil and fractions. Eur J Lipid Sci Technol. 2007;109(4):359–72.
24. Chong CL, Kamarudin Z, Lesieur P, Marangoni A, Bourgaux C, Ollivon M. Thermal and structural behaviour of crude palm oil: crystallisation at very slow cooling rate. Eur J Lipid Sci Technol. 2007;109(4):410–21.
25. Bugeat S, Briard-Bion V, Pérez P, Pradel P, Martin M, Lesieur S, Bourgaux C, Ollivon M, Lopez C. Enrichment in unsaturated fatty acids and emulsion droplet size affect the crystallization behaviour of milk triacylglycerols upon storage at 4 °C. Food Res Int. 2011;5:1314–30.
26. Ollivon M, Keller G, Bourgaux C, Kalnin D, Villeneuve P, Lesieur P. DSC and high resolution X-ray diffraction coupling. J Therm Anal Calorim. 2006;83:219–26.
27. Helgason T, Awad T, Kristbergsson K, McClements D, Weiss J. Influence of polymorphic transformations on gelation of tripalmitin solid lipid nanoparticle suspensions. J Am Oil Chem Soc. 2008;85(6):501–11.
28. Ng WL, Oh CH. A kinetic study on isothermal crystallization of palm oil by solid fat content measurements. J Am Oil Chem Soc. 1994;71(10):1135–9.
29. Tarabukina E, Jego F, Haudin JM, Navard P, Peuvrel-Disdier E. Effect of shear on the rheology and crystallization of palm oil. J Food Sci. 2009;74(8):405–16.
30. Walstra P. Secondary nucleation in triglyceride crystallization. Prog Colloid Polym Sci. 1998;108:4–8.