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Development of a Lipid Particle for β-Carotene Encapsulation Using a Blend of Tristearin and Sunflower Oil: Choice of Lipid Matrix and Evaluation of Shelf Life of Dispersions

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Summary

Solid lipid particles are colloidal carriers that have been studied for almost 20 years in the pharmaceutical field and recently have been investigated by food researchers due to their capacity to enhance the incorporation of lipophilic bioactives and their bioavailability in aqueous formulations. The aims of this study are to choose a suitable lipid matrix to produce solid lipid particles, which would be used to encapsulate β-carotene, and to evaluate the capacity of dispersions to protect the incorporated carotenoid. Bulk lipid mixtures of tristearin and sunflower oil were analysed by differential scanning calorimetry and wide angle X-ray diffraction, and the mixture with the highest degree of structural disorganisation was chosen. β-Carotene was then encapsulated in solid lipid particles produced with this mixture, composed of 70 % tristearin and 30 % sunflower oil (6 % total lipid) and stabilised with hydrogenated soy lecithin and Tween 80 (3 % total surfactant) by hot pressure homogenisation. Two types of particles were produced, using one or two passages in the homogenisation step. Average particle size, zeta potential, thermal behaviour, crystallinity and β-carotene concentration were monitored over 4 months of storage (under refrigerated conditions). The results showed minor differences between the systems in terms of size distribution, although the particles produced with one passage through the homogeniser were slightly more efficient at protecting the β-carotene from degradation and also suffered few microstructural alterations after 4 months.

Key words: solid lipid particles, β-carotene, encapsulation

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Introduction

Lipids are known to enhance oral absorption of several bioactives, especially hydrophobic ones (*1–4*). Among the hydrophobic bioactives of greatest interest in food applications is β-carotene, due to its provitamin A activity, its attributed preventive effects against some types of chronic disorders, such as cancer and cardiovascular diseases, and the enhancement of the immunological system (*5,6*). However, the high degree of hydrophobicity often significantly reduces the bioavailability of β-carotene from food formulations and also makes dispersion in aqueous media difficult. The use of lipid-based matrices (*e.g.* emulsions (micro- and nanoemulsions), liposomes and solid lipid particles for encapsulation is a proven method to overcome both problems (*7–17*). The bioavailability of β-carotene can be increased in the presence of lipids by a variety of mechanisms (*e.g.* an increase in gastric retention time, the ability of lipids to affect the physical and biochemical barrier function of the gastrointestinal tract and the stimulation of the secretion of bile salts and endogenous biliary lipids) (*18,19*).

Solid lipid particles are colloidal carrier systems similar to conventional emulsions, but with a nanometric hydrophobic core composed of a lipid solid at room temperature, stabilised by surfactants (*20*). These dispersions were designed to combine the advantages of polymeric nanoparticles, liposomes and emulsions (*16*), presenting extended shelf life, biocompatibility, a good level of protection of sensitive bioactives and controlled release (*21, 22*). There are several methods that can be employed to produce the solid lipid particles, such as the microemulsion technique, the solvent emulsification-evaporation or diffusion method, solvent injection, and water/oil/water (w/o/w) double emulsion (*22,23*). However, none of these methods has greater potential to be scaled up than high pressure homogenisation (HPH). HPH is the most common production method in the preparation of solid lipid particles and can be performed at elevated temperature (hot HPH) or below room temperature (cold HPH) (*21,22,24*). Hot HPH is more often found in the literature than other methodologies, even in the case of thermolabile bioactives, as the exposure to an increased temperature is relatively short. Normally, hot coarse emulsions are produced by high speed agitation in a high-shear mixer (*e.g.* Ultra-Turrax®, IKA®, Staufen, Germany), and afterwards processed in a high pressure homogeniser set above the melting temperature of the lipids, in which more than one passage through the homogeniser can be used. The number of passes is a process condition that can strongly influence the characteristics of the produced dispersions, especially the particle size distribution, but the exposure of a sensitive bioactive to a high temperature for a longer period can be critical for its stability during the storage (*13,25*).

After the completion of HPH, the fine emulsion is cooled to crystallise the particle core. This final step is critical as the types of crystals formed are extremely dependent on the rate of cooling of the dispersions and the type of surfactant used to stabilise the nanodispersions (*22,23*).

Crystallinity and polymorphic behaviour are crucial properties of the solid lipid particles. The crystalline structure is intimately related to the chemical nature of the lipid and is a key factor in the phenomenon of incorporation or expulsion of the bioactive from the particles (*22,23,26*). Theoretically, the more disorganised the crystalline structure is, the higher the particle capacity to incorporate bioactive molecules will be, as microstructural disorganisation results in the presence of 'voids' capable of accommodating a higher amount of the encapsulated substance. This approach can be achieved by mixing solid lipids with liquid lipids, instead of highly purified lipids with relatively similar molecules.

Therefore, it is important to know the thermal and crystalline characteristics of a new lipid matrix when determining its suitability for use in a certain application (*27*). Using techniques such as DSC (differential scanning calorimetry), WAXD (wide angle X-ray diffraction), IHCMC (isothermal heat conduction microcalorimetry) and TEM (transmission electron microscopy) (*24,27–30*), the potential of a lipid matrix to be employed in a lipid particle can be determined.

Based on these factors, this research was conducted to characterise mixtures of tristearin and sunflower oil with the intention of applying their blends in food applications, such as solid lipid particles. Long-chain saturated fatty acids are generally believed to increase serum cholesterol levels. However, stearic acid is neutral with respect to cholesterol levels in the blood because its melting point is much higher than body temperature and because it is readily desaturated to oleic acid *in vivo* (*31, 32*). Mixtures of sunflower oil and tristearin were produced and analysed by DSC and WAXD to determine the best combination for the production of lipid particles. The colloidal dispersions encapsulating β-carotene were produced by hot pressure homogenisation, under different operational conditions, and the shelf life of the particles was evaluated. The evaluation was based on the capacity to preserve the encapsulated carotenoid, and the ability to maintain the average particle size, zeta potential and microstructure. The microstructures of the lipid particles were analysed by thermal behaviour and crystalline structure at the time of production (fresh samples) and over a storage time of 4 months. The choice of a mixture of saturated hydrogenated phospholipid and polysorbate 60 was due to the fact that the use of the phospholipid alone could lead to the formation of highly viscous or gel-like systems. In addition, the stabilisation with this food-grade emulsifier may have the advantage of slowing down possible polymorphic transitions in lipid particles (*33–35*).

Materials and Methods

Materials

β-Carotene, tristearin and polysorbate 60 (Tween 60) were obtained from Sigma (St. Louis, MO, USA), sunflower oil was obtained from Cargill (Mairinque, Brazil), and purified and hydrogenated soy lecithin was obtained from Lipoid (Lipoid S100-H, Ludwigshafen, Germany). All reagents employed in the study were of reagent grade. Deionised water (from a Millipore system, Millipore, Billerica, MA, USA) was used throughout the experiments.

Production of bulk lipid matrices

The bulk, solid lipid (tristearin) and binary mixtures (tristearin and sunflower oil) were analysed separately. The lipid mixtures contained 10 and 30 % sunflower oil (by mass) with the remaining mass attributed to tristearin. Fusion consisted of melting in a thermostatic water bath at 80 °C. After the fusion, the samples were kept at room temperature until they had completely cooled and solidified.

Production of lipid particles

Lipid particles (LP) were produced by melting the lipid phase (30 % sunflower oil and 70 % tristearin) at 80 °C, and adding β-carotene to the melted lipid mixture, according to the formulations described in Table 1. The carotenoid was added to the lipids after melting to minimise degradation by high temperature. The hot surfactant solution at 80 °C was mixed with the melted lipid and submitted to ultra-agitation (9000 rpm for 3 min, in an IKA® T25 homogeniser, IKA®, Staufen, Germany). Afterwards, the coarse emulsions were subjected to the high pressure homogenisation step (Panda 2K homogeniser, NS1001L, GEA Niro Soavi, Parma, Italy). The emulsions were passed from one to three times through the homogeniser, at 80 °C, under the process conditions presented in Table 1. Lipid particles were produced in triplicate and stored under refrigerated conditions (7–10 °C, protected from exposure to light and under unmodified atmosphere).

Determination of average particle size (hydrodynamic diameter), particle size distribution and zeta potential

The average particle size (hydrodynamic diameter) and size distribution of the lipid particles were obtained by photon correlation spectroscopy, using a 90Plus particle size analyser (Brookhaven Instruments Corporation, Holtsville, NY, USA) at 25 °C and a He-Ne laser at 627 nm with an incidence angle of 90°. The samples were diluted with ultra-purified water to weaken the opalescence before measuring the mean particle diameter.

The zeta potential was obtained using a ZetaPALS ζ-potential analyser (Brookhaven Instruments Corporation) in purified water, adjusting the conductivity to 50 μS/cm, and was calculated from the electrophoretic mobility using the Helmholtz–Smoluchowski equation. Data analyses were performed using the software included with the system. Both the hydrodynamic diameters and zeta potential were expressed as the mean value of ten measurements performed by the equipment, for each of the three dispersions produced.

Differential scanning calorimetry

The thermal analyses by differential scanning calorimetry (DSC) of both bulk lipid mixtures and lipid particles were conducted at a ramp rate of 10 °C/min, in the range of 0 to 100 °C, using a TA5000 calorimeter (TA Instruments, New Castle, DE, USA). An empty aluminium pan was used as a reference. The crystallinity index (CI) was calculated from the heat of melting using the equation:

$$\mathrm{CI}/\% = \frac{\text{enthalpy LM}}{\text{enthalpy PL}} \cdot \mathrm{fM} \cdot 100 \qquad /1/$$

where enthalpy LM is the melting enthalpy of the lipid mixture, enthalpy PL is the melting enthalpy of the pure lipid, and fM is a factor that accounts for the concentration of the solid lipid in the matrix (*27–30*).

Wide-angle X-ray diffraction

Wide angle X-ray diffraction (WAXD) analyses were conducted in a Philips PW1710 X-ray diffractor (Phillips, Eindhoven, The Netherlands), with a copper anode that delivered an X-ray wavelength (λ) of 1.5418 Å. The measurements were obtained in the range of 2θ from zero to 40° using steps of 0.02° for both the lipid mixtures and the solid lipid particles, at room temperature. The interlayer spacings were calculated using Bragg's law:

$$d = \frac{2\lambda}{\sin\theta} \qquad /2/$$

where d is the interplanar spacing in the atomic lattice, λ is the wavelength of the incident X-ray beam, and θ is the scattering angle (in radians).

Determination of β-carotene content in the lipid particles

Encapsulated β-carotene was determined using spectrophotometry. The carotenoid was extracted with hexane after destabilisation of the nanoparticulate system with sodium chloride (protocol adapted from Hentschel *et al.* (*16*)). Ultrasound was used to assist in the destabilisation of the dispersion and in the partitioning of the β-carotene to the hexane phase. After β-carotene extraction, the absorbance of the organic phase was read at 450 nm. The measurements were carried out in triplicate

Table 1. Formulations of β-carotene-loaded lipid particles (LP) (in percentage of the total mass of the produced dispersions)

Particle	*w*(lipid)=6 %	*w*(surfactant)=3 %	*w*(β-carotene)/%*	*p*(homogenisation)/kPa	HPH step
	w/%				
LP-1	4.2 TS 1.8 SO	2.4 SL 0.6 PS60	0.030	$5 \cdot 10^4$	1
LP-2	4.2 TS 1.8 SO	2.4 SL 0.6 PS60	0.030	$5 \cdot 10^4$	2

*value based on Hentschel *et al.* (*16*)
TS=tristearin, SO=sunflower oil, HPH=high pressure homogenisation, SL=soy lecithin, PS60=polysorbate 60
Deionised water was added to the samples up to 100 mL

for each sample, and the β-carotene was quantified according to a calibration curve of pure β-carotene in hexane, with an R^2 of 0.999.

Determination of colorimetric parameters

The degradation of β-carotene was also monitored by instrumental colorimetry. The parameters of the tristimulus colour system ($L^*a^*b^*$) were obtained using a colorimeter (Miniscan XE, HunterLab, Hunter Associates Laboratory, Inc., Reston, VA, USA). The parameter colour change (ΔE^*) was then calculated as follows:

$$(\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}) \qquad /3/$$

Dispersions of LP were placed in a transparent flat-faced cuvette, and the measurements were obtained after the colorimeter had been pressed against the cuvette surface. All measurements were repeated three times.

Results and Discussion

Analysis of the bulk lipid matrices containing tristearin and sunflower oil

The WAXD data showed pure tristearin (TS) reflections typical of β arrangement (at 0.42 and 0.38 nm), as shown in Fig. 1. However, the presence of sunflower oil modified the structure of pure solid triacylglyceride significantly, causing three effects: (*i*) the decrease of both peak intensities, (*ii*) broadening of the peaks, and (*iii*) the appearance of a peak attributed to β phase (at 0.46 nm), which is a clear indication that the liquid oil was able to strongly alter the organisation of the lipid matrices.

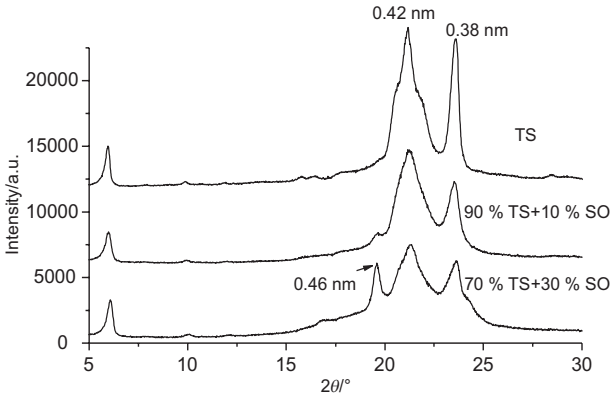

Fig. 1. WAXD analyses of bulk lipid mixtures of tristearin and sunflower oil
TS=tristearin, SO=sunflower oil

The DSC data presented in Table 2 also confirm the alterations in the lipid matrices due to the addition of sunflower oil (SO). The crystallinity indices of samples with TS+SO indicated a much less organised structure than in pure TS, despite the presence of the β phase.

Sunflower oil is a raw material composed of triacylglycerols containing oleic and linoleic acids in their structures (*36*). Therefore, its addition to the tristearin would be responsible for the formation of much less-ordered matrices containing imperfections in their structures. In this study, the calorimetric data suggest that the addition of 30 % sunflower oil significantly altered the structure of the lipid matrix in comparison with the mixtures composed of 10 % liquid lipid, as the CI of 50.6 % in the mixtures containing 30 % sunflower oil confirmed the existence of a much lower degree of crystal structure order. Such a disorganization was also confirmed by a quite large decrease in the melting enthalpies (ΔH_m), from a ΔH_m equal to 157.3 J/g for the pure tristearin to 113.8 J/g for the mixtures with 30 % of sunflower oil (*i.e.* a decrease of almost 30 %). Thus, this mixture was chosen to produce the β-carotene-loaded lipid particles.

Production, characterisation and shelf life of β-carotene-loaded lipid particles

Two process parameters were optimised to obtain the desired characteristics of the dispersions: homogenisation cycles and pressure of operation. Pressures varying from $350 \cdot 10^2$ to $1000 \cdot 10^2$ kPa were tested. The lowest pressure tested ($350 \cdot 10^2$ kPa) resulted in dispersions with a lower hydrodynamic diameter (160 nm) and monomodal distribution, but the dispersions were quite prone to flocculation. Higher homogenisation pressures ($700 \cdot 10^2$ and $1000 \cdot 10^2$ kPa) also resulted in phase separation after a few days of storage under refrigeration (data not shown). In all tests, independent of the pressure, the use of 3 passages led to the formation of high flocculating systems. The size distribution curves show that the process of homogenisation at $500 \cdot 10^2$ kPa produced particles within the nanometric range, but in bimodal distribution. Typical size distribution curves for fresh samples of LP produced under this condition, with 1 or 2 passages in the HPH, are shown in Fig. 2.

Therefore, the conditions suitable for the production of particles were the pressure of $500 \cdot 10^2$ kPa and 1 or 2 passages (LP-1 and LP-2) through homogeniser, because under these conditions particles with average diameter of approx. 300 nm were produced, even though their distribution was not monomodal. The dispersions produced using one passage (LP-1) had only a small population of particles with average size of 120 nm. The aver-

Table 2. Calorimetric data obtained by differential scanning calorimetry for the lipid mixtures of tristearin and sunflower oil

Formulation	T_{onset}/°C	$T_{melting}$/°C	ΔT/°C	$\Delta H_{melting}$/(J/g)	CI/%
Pure TS	59.1	61.8	2.7	157.3	–
90 % TS+10 % SO	57.8	61.5	2.8	148.7	85.1
70 % TS+30 % SO	56.5	60.2	3.7	113.8	50.6

TS=tristearin, SO=sunflower oil, CI=crystallinity index

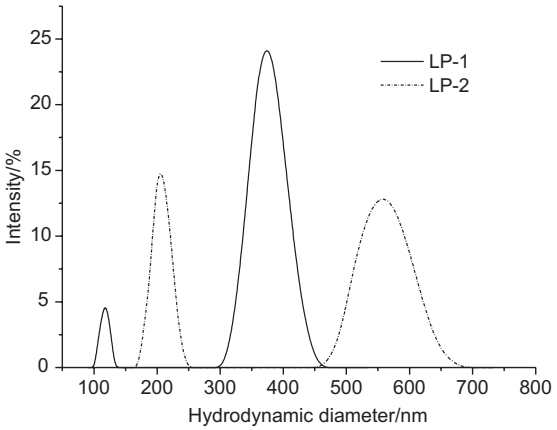

Fig. 2. Typical size distribution curves of the dispersions of lipid particles (LP) produced with tristearin and sunflower oil

age hydrodynamic size of LP-2 was very similar to LP-1, although the distribution was different, with two populations with average sizes of 200 and 550 nm.

As for the stability of the dispersions during storage, Fig. 3 shows the variation in mean hydrodynamic diameter and zeta potential.

The capacity of the LP to protect β-carotene from degradation during storage is shown in Fig. 4.

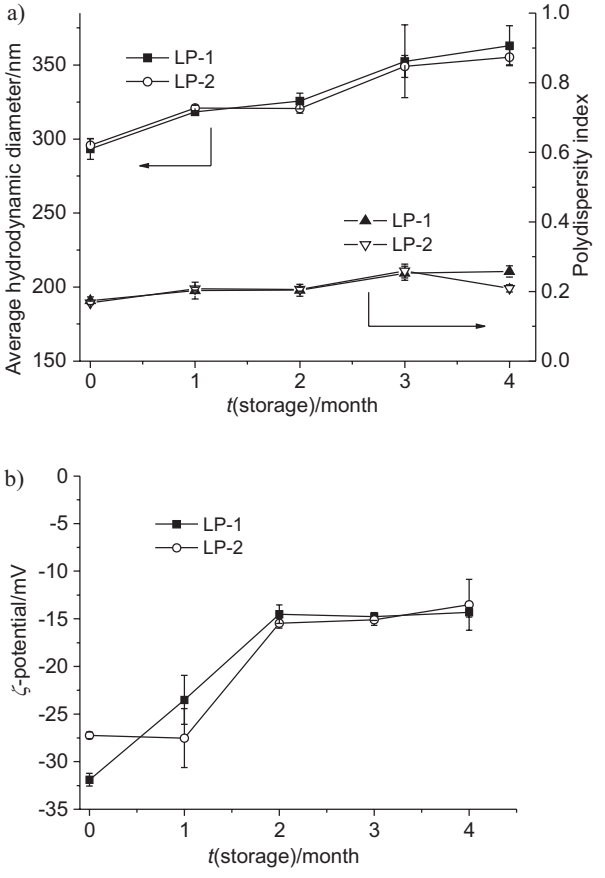

Fig. 3. Characteristics of the lipid particle (LP) dispersions over the storage period: a) average hydrodynamic diameter and polidispersity index, and b) zeta potential

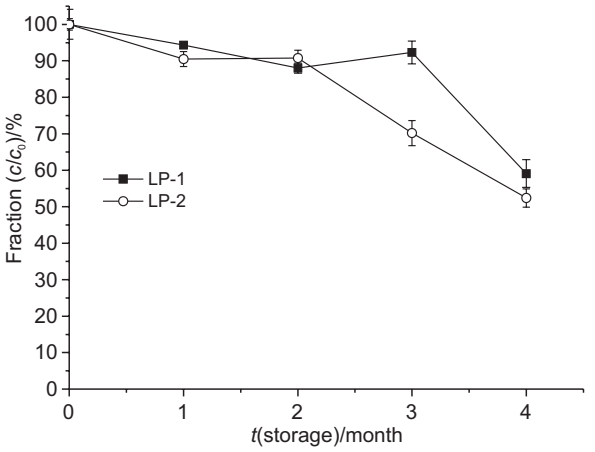

Fig. 4. Degradation of β-carotene in the lipid particles (LP) when stored under refrigeration for 120 days. c_0 is the β-carotene concentration in fresh samples, and c is the concentration of the carotenoid in the particles at the time of sampling (samples were prepared in triplicate)

The lipid particles produced using one passage through the HPH were more efficient in the protection of the carotenoid. While LP-2 preserved 90 % of the initial amount of β-carotene for 2 months, LP-1 preserved the same amount up to three months. A difference of 30 days between samples in the start of a more intense decay in the β-carotene concentration can be attributed to the slightly longer period of exposure of LP-2 to high temperature during the second passage through the HPH. This is likely due to the formation of a higher amount of free radicals during the homogenisation step in the production of LP-2. The same conclusion was reached by the authors in a study about the influence of the number of passages in the oxidation of flaxseed oil emulsions (*25*). They observed an increase in the concentration of primary oxidation products in emulsions produced by HPH at higher temperatures and pressures. A similar process occured in our LP-2 system, where the production temperature was 80 °C, and the exit temperature was higher.

In addition, if the results obtained are compared with similar studies found in the literature, it is noted that both LP-1 and LP-2 systems performed well when compared to other nanostructured systems encapsulating β-carotene. Many studies investigating the stability of β-carotene encapsulated in nanoemulsions (*10*,*11*,*13*, *14*,*18*) test a great variety of lipids and surfactants, and most of these studies do not evaluate shelf life longer than 30 days, while ours monitored the nanosystems for 4 months (120 days). The results in the literature vary greatly in terms of capacity of protecting β-carotene against degradation, ranging from 10 to 50 % degradation of the initial amount of encapsulated carotenoid. Our results showed that it is possible to preserve β-carotene in the nanoparticles for over 90 days of storage, without using any antioxidant to protect it against oxidation.

Our results agree with similar studies testing the capacity of solid lipid particles to avoid the degradation of β-carotene (*9*,*16*,*37*–*39*). Hentschel *et al*. (*16*) encapsulated β-carotene in solid lipid particles of propylene glycol

monostearate stabilised with Tween 80. The authors detected aggregation of the particles stored at low temperature (4–8 °C) for 30 weeks. They also determined that the presence of α-tocopherol was necessary to preserve the encapsulated β-carotene.

Using hydrogenated palm kernel oil (HPKO) and palm glycerides, and a mixture of Span 40 and Tween 80 as emulsifiers, Hung *et al.* (*37*) produced solid lipid carriers by HPH using three different methods, which were capable of preserving nearly 100 % of the initial β-carotene content after 90 days of storage (when tocols were present as protecting agents). The authors observed that when the tocols were degraded, the particles were not stable in terms of average size, and they attributed this finding to the alteration of the size and the formation of oxidation products, which could have reacted with the surrounding interfacial membrane causing the droplets to coalesce. A similar phenomenon may have occurred in our study, explaining the modifications in zeta potential and average hydrodynamic diameter (both shown in Fig. 2).

Conversely, Helgason *et al.* (*15*) showed that pure tripalmitin nanoparticles stabilised with polysorbate 80 were not effective at protecting encapsulated β-carotene. Their system was composed of only one type of solid triacylglycerol, which probably caused crystallisation from the interior to the surface, causing β-carotene to be expelled from the highly organised crystal subcells. As a result, the encapsulated bioactive molecules were forced onto the droplet surface in closer proximity to prooxidants in the aqueous phase.

In regard to this particular aspect of crystallisation, Tikekar and Nitin (*40*) studied the influence of physical state (solid and liquid) of the lipid core on the rate of oxidation in lipid nanostructures and demonstrated that the rate of oxygen and peroxyl free radical transport was not significantly affected by the physical state of the structure. Rather, they proved the phenomenon of expulsion of the encapsulated molecules. This is a strong indication that the lipid matrices must be investigated before chosing one, so that the formation of extremely organised crystals is avoided. This minimises the expulsion of the bioactive to the surface of the droplets, and consequently, their exposition to prooxidant agents. Such an expulsion of β-carotene to the surface was likely reduced in the present study because the particles were produced with a mixture of lipids, resulting in a highly disorganised particle structure, as indicated by DSC and WAXD results, which in fact formed a blend.

Colour measurements are a complementary method to evaluate the chemical stability of encapsulated β-carotene in the LP (*10*). The variations in the CIELAB parameters a^*, b^* and L^*, and the total colour difference (ΔE^*) are shown in Fig. 5.

The data in Fig. 5 completely agree with the β-carotene quantification data in Fig. 4. The data obtained for colorimetric parameters indicated that the colour fading up to the 2nd month for LP-1, and 3rd month for LP-2, was minimal. The values of b^*, which is an indication of the intensity of yellowness of the samples, showed decay after 2 months of storage, as expected due to the tendency of degradation of β-carotene, especially for

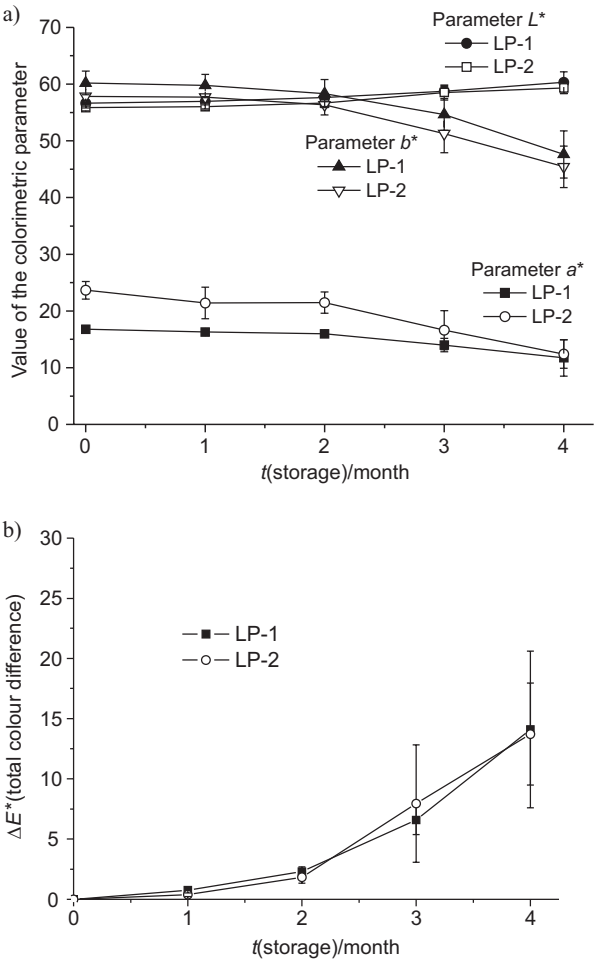

Fig. 5. Colorimetric parameters over the storage period for the lipid particles (LP): a) parameters a^*, b^* and L^*, and b) representation of the colour fading by the parameter colour difference (ΔE^*)

LP-2. The values of lightness (L^*) increased slightly after 2 months of storage, which is in agreement with the degradation of β-carotene, resulting in samples turning whiter. The values of the parameter ΔE^* (total colour difference) were also in agreement with the temporal profile shown in Fig. 4 (it increased after the 2nd for LP-1 and 3rd month for LP-2). Such results are important, as the lack of sensorial alterations in food ingredients over the storage period is one of the most critical requirements.

Regarding the structural analysis of the β-carotene-loaded lipid particles, Fig. 6 shows the diffractograms obtained by WAXD for fresh and stored samples.

The diffractograms were obtained for fresh and 120-day-old samples of LP-1 and LP-2. Peaks occur at 0.48, 0.43 and 0.40 nm, and also a shoulder at 0.37 nm, for both fresh particles. None of these peaks corresponds to the expected spacing for the typical polymorphs of triacylglycerols (0.46 nm for α, 0.42 and 0.38 nm for β' and 0.46 nm for β) (*41,42*). However, the LP-1 and LP-2 were produced with a mixture of tristearin (saturated triacylglycerol) with sunflower oil (a mixture of triacylglycerols). The main types of triacylglycerols found in the SO are formed by mixtures of linoleic and oleic chains (Ln-

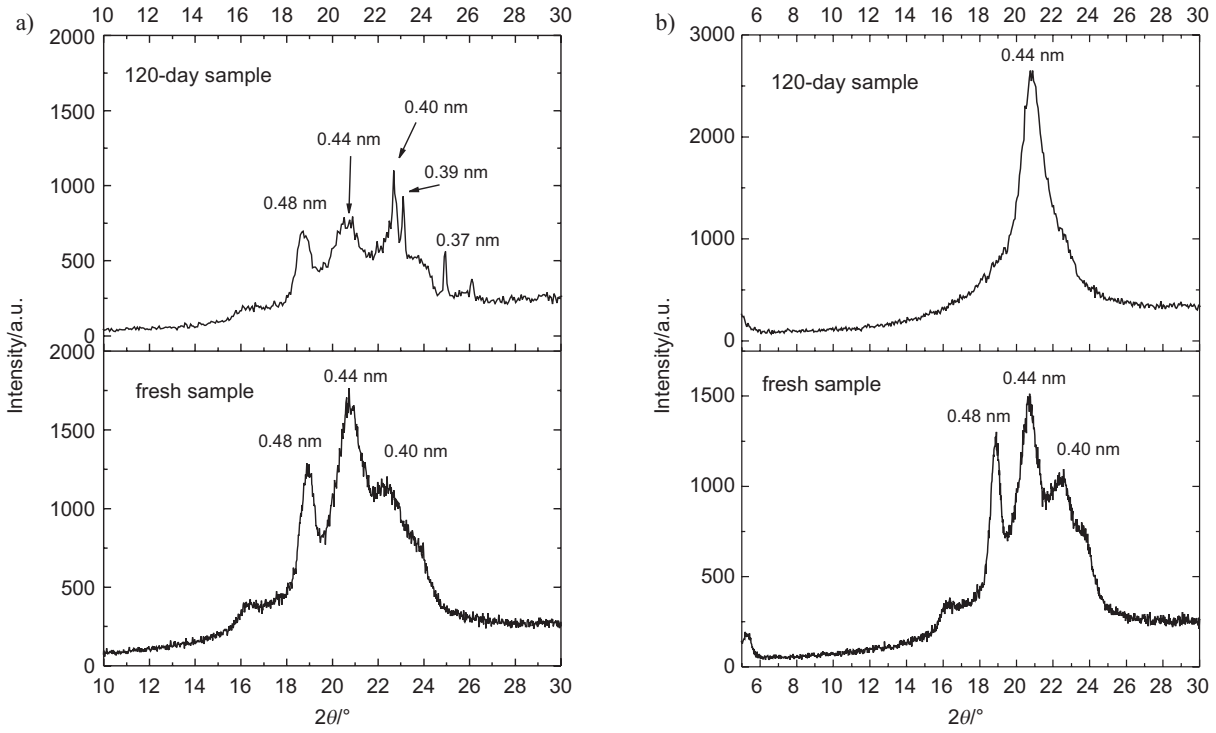

Fig. 6. X-ray diffractograms obtained by WAXD of the β-carotene-loaded lipid particles (LP): a) LP-1, and b) LP-2

LnLn, OLnLn, OLnO or OOLn, and PLnO, where P=palmitic acid, Ln=linoleic acid and O=oleic acid) (*36*,*43*). It was expected that the presence of 30 % SO would significantly alter the structure of the pure crystals of TS, resulting in forms like sub-β. Timms (*42*) defines this sub-β as a form that does not satisfy the criteria for α and β, showing a strong spacing at approx. 0.474 nm and several strong spacings at approx. 0.45, 0.39 and 0.36 nm. These data seem to fit exactly the structure indicated by diffractograms for fresh particles in Fig. 6.

However, after 4 months of storage, different patterns of WAXD were observed for LP-1 and LP-2. The particles that passed only once through the HPH presented a crystalline structure which produced diffraction peaks at 0.48, 0.43, 0.40, 0.39 and 0.37 nm, similar to the diffraction pattern of fresh samples. However, the LP-2 sample presented just one peak at 0.43 nm after 120 days of storage. The peak was quite broad and noticeable, indicating the samples were very amorphous. However, such a broadening of the peak may have hidden other peaks, presenting a false impression that it was the only characteristic dimension existent in the crystalline structure after 4 months of storage. Regardless, a rearrangement of the structure occurred, and it may be related to the degradation of β-carotene after the 2nd month of storage, which was clearly more pronounced for LP-2, according to Fig. 4.

Finally, Fig. 7 shows that the thermal behaviour of the particles did not change after storage under refrigeration for 120 days. Two endothermic peaks appeared for both LP-1 and LP-2. The peak at 65 °C was likely caused by the melting of the sub-β crystals. However, the endothermic peak at 55 °C could not be attributed to the presence of α polymorph, as it was not evident in the WAXD data. No spacing at 0.46 nm was present in the crystalline structure of any of the LP, indicating that the transition at 55 °C was not caused by the melting of α-crystals. There are two possibilities for this phase transition: (*i*) it was due to emulsifier interactions at the interface of the particles (*9*,*15*), or (*ii*) due to the presence of liposomes formed during the high pressure homogenisation. The latter would be possible because the liposomes, if present, would have been composed of Lipoid S-100-H, whose gel-liquid crystalline transition occurs at 55 °C (*44*).

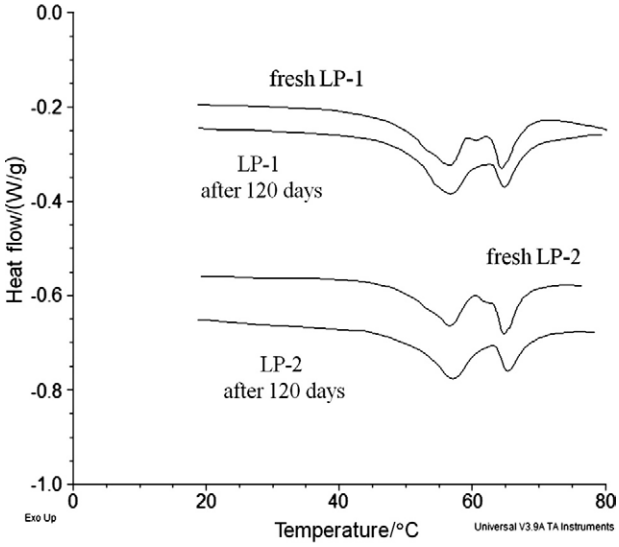

Fig. 7. Thermograms obtained by DSC of the lipid particles (LP) encapsulating β-carotene (fresh samples and samples after 120 days of storage)

The absence of significant changes in the thermal behaviour of LP-2 reinforces the hypothesis that the disappearance of other peaks in WAXD diffractograms was due to the broadening of the peak at 0.43 nm, and not due to the absence of other diffraction peaks.

Conclusions

The data obtained from the DSC and WAXD were used to analyse the microstructure of tristearin and sunflower oil lipid mixtures and were extremely valuable in the evaluation of the usefulness of the proposed solid lipid and its mixtures for the production of lipid particles. Using a mixture of 70 % tristearin and 30 % sunflower oil (the mixture which presented a higher degree of microstructural disorganisation), it was possible to produce solid lipid particles using $500 \cdot 10^2$ kPa and one or two passages through the HPH.

Both LP-1 and LP-2 particles exhibited similar behaviour during storage. Both were quite stable for a relatively extended shelf life, considering they were not monomodal dispersions at the time of production. Theoretically, bimodal dispersions would have been more prone to destabilisation in terms of average size, which did not occur after 120 days of storage. The increase in zeta potential values occurred up to the 2nd month of storage, but by the 4th month, they had not suffered additional alterations, stabilising at about –15 mV.

However, in regards to the preservation of β-carotene, LP-1 seemed to be more capable of protecting the bioactive against degradation, an important characteristic which was certainly caused by the shorter time of exposure of the dispersion to the high process temperature. Besides, LP-1 dispersions were apparently less prone to crystalline structural rearrangements, according to WAXD data (a fact which can also be due to such a longer period of exposure to high temperature). However, thermal analyses did not show any significant changes after 120 days of storage, indicating that the microstructure may have been altered, but only to a small extent. The data obtained here indicate, therefore, that solid lipid particles produced with tristearin and sunflower oil can be an interesting alternative to encapsulate β-carotene and to be incorporated in food formulations, and that the number of passages is a critical process parameter for the preservation of the encapsulated bioactive.

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