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Highly Concentrated Emulsions Containing High Loads of Pterostilbene

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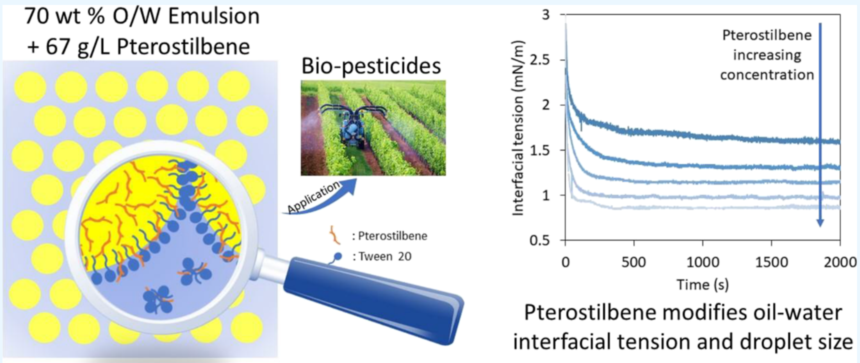

ABSTRACT: Pterostilbene is a highly researched molecule due to its bioactivity. However, its hydrophobicity limits its application. For this reason, researchers have sought to encapsulate pterostilbene (namely, in oil-in-water emulsion) to increase its availability. Studies are lacking when it comes to the effects of pterostilbene and its concentration at the oil/water interface. This paper discusses the effects of oil types, storage temperature, and pterostilbene concentration on the stability of the emulsions, as well as the interactions between encapsulated pterostilbene and the oil and water phases. Results showed that pterostilbene is present at the oil/water interface, affecting the interfacial tension and consequently the droplet size. It was also shown that encapsulation efficiency is affected by the storage temperature and oil type. Finally, it was proven that, according to oil types and storage temperature, the stability of pterostilbene to light is affected.

1. INTRODUCTION

Pterostilbene (tran-3,5-dimethoxy-4′-hydroxystilbene), noted hereafter Pts, is a stilbenoid found naturally in the leaves of grapevine, sandalwood, and heartwood, as well as in blueberries, bark of Guibourtia tessmannii, grapes, and Vaccinium berries.[1−8] Some of the bioactivities associated with it exhibit antioxidant, anti-inflammatory, antiaging, anticancer, antitumorigenic, cardio-protective, antidiabetic, and neuroprotective properties.[1−8] However, it remains a difficult molecule to handle due to its hydrophobicity and sensitivity to light and air. More specifically, Pts oxidizes turning trans-Pts into cis-Pts, the latter being an isomer with reported high bioactivity.[5,9]

A quick literature search (Scopus) shows that in the last 22 years, about 1300 studies were done on Pts, 800 of which in the last 5 years alone. The main field of studies remains in biology, pharmacology, and medicine. However, the literature available is limited when searching for publications that have specifically studied the encapsulation of Pts. Table 1 focuses on these specific articles and details the findings of these studies: the type of encapsulating system and the main reported properties (size amount of Pts if available). In most of these studies, the particles encapsulating Pts have a size in the nanometric range, to improve bioavailability, and the concentrations of Pts encapsulated are low. Only one study reported that Pts is most soluble in medium chain triglyceride (MCT) oil, prepared emulsions containing 10.4% Pts of the total emulsion, and the antioxidant activity and bioavailability of Pts were evaluated.[7] Emulsions with high concentrations of Pts and high oil fractions are important for applications like biopesticides where the goal is to formulate a concentrated emulsion that will be diluted by the farmer just before application. Since the dilution has to be in water, stable highly concentrated oil-in-water emulsions are needed. Furthermore, nanometric oil droplet sizes are not required for this kind of application, which make the encapsulation techniques reported in the literature not relevant.

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Table 1. Literature Review on Pts Encapsulation: the Table Summarizes the Kind of System Used To Encapsulate Pts as well as the Main Reported Properties

type of system	oil type	oil fraction (%)	Pts concentration	stabilizer	energy input technique	minimum droplet size	polydispersity index	ref
O/W emulsion	medium chain triglyceride	10	1% in oil phase	sodium caseinate	high-pressure homogenizer	≈250 nm		1
	sunflower							
	olive							
microspheres			in microspheres (0.1 w/w%)	chitosan−vanillin microspheres	stirring	5−25 μm		2
nanospheres			in nanospheres (0.02 w/v%)	zein-fucoidan	stirring	120−140 nm	monomodal	3
O/W emulsion	flaxseed	4	2 w/v% in oil phase	Tween 20	high-pressure homogenizer	360 nm	monomodal	4
	olive					365 nm		
O/W emulsion		10.5	1.5 wt % in oil phase	polyoxyethylenated castor oil	phase inversion	55.8 nm	monomodal	5
liposomes			in liposomes (0.1w/w %)	DOTAP-cholesterol	sonication	435 nm	0.5	6
nanospheres			1:5 ratio in zein nanospheres	zein	ultrasound	104 nm	0.16	8
O/W emulsion	medium chain triglyceride	50	20.9 wt % in oil phase	lecithin	high-pressure homogenizer	212 nm		7

To the author's knowledge, no studies have been carried out studying the effects of the ingredients used to prepare the emulsions, the interactions between these ingredients and Pts, and the concentration of Pts on the stability of emulsions and encapsulation efficiency. This kind of study is crucial to detect the stability limits of Pts-concentrated emulsions.

The aim of this study is therefore to produce concentrated simple oil-in-water emulsions to encapsulate Pts. The kinetic stability of these emulsions has been studied as a function of the choice of the oil phase that can be either solid or liquid, the concentration of encapsulated Pts, and the effect of light on Pts. Also, herein the interaction between Pts and the oil phase and the emulsifier will be discussed.

2. MATERIALS AND METHODS

2.1. Materials. For the emulsions, medium chain triglyceride (MCT) oil (Labrafac CC) was gratefully provided by Gattefossé SAS (France), and coconut oil, vitamin E (α-tocopherol), and Tween 20 were purchased from Sigma-Aldrich. Pts was purchased from iChemical (China).

2.2. Emulsification Process. All the emulsions that were prepared were simple oil-in-water emulsions. They were stabilized by Tween 20 (25 wt % of the aqueous phase corresponding to 7.5 wt % with respect to the total emulsion), and the oil fraction was 70 wt % of the total emulsion. The oils used were coconut oil (crystallization temperature: 24 °C) or MCT oil (crystallization temperature: −2 °C). Pts was dissolved in the oil phase (in aluminum-wrapped containers to limit Pts photodegradation) the day prior to emulsification and left stirring overnight at 30 °C for MCT and coconut oil. Emulsions (30 g) were produced (in aluminum-wrapped containers) using a high shear homogenizer (ULTRA TURRAX, IKA T25) at 8800 rpm for 1 min and then split into two equal samples; one to be stored at 27 °C (above the crystallization temperature) and the other at 4 °C. Table 2 lists the different concentrations of Pts tested in the emulsions, and all the Pts concentrations reported in this paper are concentrations in the pure oil phase. All the emulsions were produced in duplicates.

2.3. Emulsion Characterization. *2.3.1. Droplet Size Distribution.* After the formation of the emulsion, a sample was collected and diluted 20 times (~3.5 wt % of oil) with distilled water and used for droplet size and droplet size distribution measurements. Due to the presence of enough surfactant, this dilution of water did not alter the droplet size distribution. The measurements were done using a laser diffraction equipment (Malvern Mastersizer 2000), and the Sauter mean diameter ($d_{3,2}$) and span values were reported. The Sauter mean diameter is defined by eq 1a:

$$d_{3,2} = \frac{\sum_{i-1}^{n} n_i D_i^3}{\sum_{i-1}^{n} n_i D_i^2} \quad (1a)$$

where n_i and D_i are the number and diameter of droplets in a particular size fraction, respectively. It corresponds to the surface-average diameter. The span is defined as:

$$\text{span} = \frac{(d_{90} - d_{10})}{d_{50}} \quad (1b)$$

where d_{90}, d_{50}, and d_{10} are the diameters corresponding to a cumulative volume of 90, 50, and 10% of the total dispersed phase volume, respectively.

Table 2. Composition of the Simple Emulsions

oil phase	oil fraction (wt %) with respect to the total sample	emulsifier fraction (wt %)	Pts concentration (g/L) in oil	Pts concentration (g/L) in emulsion
coconut	70	25% Tween 20 with respect to the aqueous phase = 7.5% with respect to the total emulsion	0	0
			19.7	14.2
			29.3	21.2
			38.7	28.2
			48.0	35.0
			57.1	41.8
			66.1	48.4
			75.0	55.0
			92.3	68.0
			109.1	80.7
MCT	70	25% Tween 20 with respect to the aqueous phase = 7.5% with respect to the total emulsion	0	0
			36.9	27.8
			104.2	79.6

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Table 3. Parameters for Pts Stability to UV Light

		medium	concentration of Pts (g/L)	storage light	storage temperature (°C)	oil state
effect of Pts concentration		ethanol	0.55	dark	27	no oil
				UV lamp		
effect of oil type		coconut oil emulsion	87	UV lamp	4	crystalline
		coconut oil			4	crystalline
		coconut oil			27	liquid
		MCT oil emulsion			27	liquid
		MCT oil			27	liquid
vitamin E concentration						
effect of vitamin E	0.015 wt % of oil phase	ethanol	0.55	UV lamp	27	
	10 wt % of oil phase	coconut oil emulsion	87		4	crystalline
		coconut oil			4	crystalline
		coconut oil			4	crystalline

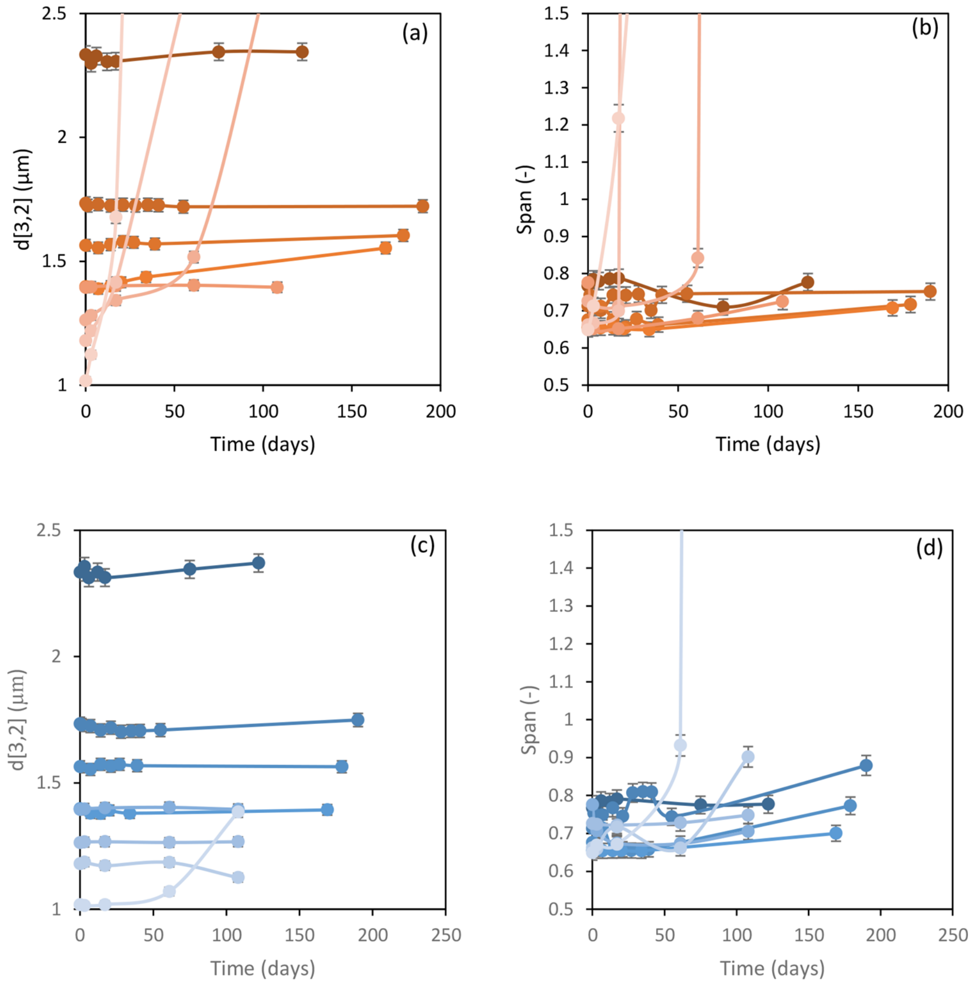

Figure 1. Droplet size and droplet size distribution over time of 70% coconut oil-in-water emulsions stabilized by Tween 20, containing 0, 19.7, 29.3, 38.7, 48.0, 57.1, 66.1, and 75.0 g/L Pts (from darkest to lightest color gradients) and stored at 27 °C (a, b) and 4 °C (c, d). Lines are guides for the eyes and not a curve fitting.

Over time, the oil droplets' size was monitored by homogenizing the emulsion, collecting a sample, and following the same dilution and measurement procedure just described.

2.3.2. Encapsulation Efficiency. Spectroscopy (JASCO V-730) was used to quantify the encapsulated concentration of Pts defined as the ratio of Pts inside the oil drops to the total amount of Pts added. Absorbance was measured at 320 nm. A coconut oil−Pts solution was prepared at 0.1 wt % Pts and then diluted in pure ethanol to obtain 0−0.002 wt % Pts solutions. A concentration standard curve was plotted (Figure S1 in the Supporting Information) to obtain eq 2:

$$\text{absorbance} = 950.26 \times \text{concentration (\%)} \quad (2)$$

This equation is the result of three independent replicates (R^2 = 0.996). Note that at a wavelength of 320 nm, neither ethanol nor coconut oil interferes with the absorbance of Pts.

To measure the encapsulation efficiency in the emulsions, 1.9 g of a stable emulsion was loaded into an Optima MAX-XP

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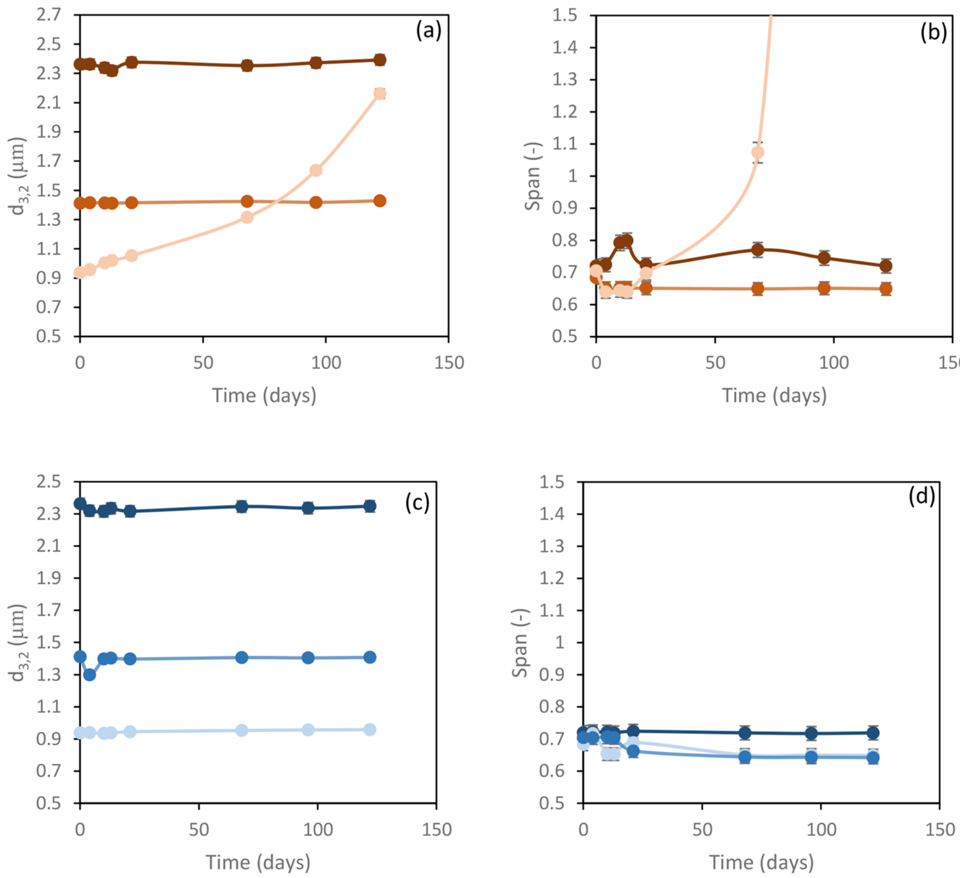

Figure 2. Droplet size and droplet size distribution over time of 70% MCT oil-in-water emulsions stabilized by Tween 20, containing 0, 36.9, and 104.2 g/L Pts (from darkest to lightest color gradients) and stored at 27 °C (a, b) and 4 °C (c, d). Lines are guides for the eyes and not a curve fitting.

ultracentrifuge and centrifuged (in 7 × 20 mm tubes) at 53,000 rpm for 40 min at 30 °C to break the emulsion. A sample of the oil phase on top was collected, diluted in ethanol (to a value that lies in the standard curve shown in Figure S1), and, using eq 2, the concentration of Pts was calculated. If 100% of the Pts initially dissolved in the oil phase is detected by spectroscopy, this means all the Pts molecules remained in the oil phase; otherwise, a part has migrated to the aqueous phase.

2.3.3. Pts Stability to UV Light. Since Pts is sensitive to UV light, several tests were set up to determine its stability in the emulsions. The studied variables were the effect of Pts concentration, oil type, and finally the effect of adding a natural antioxidant (vitamin E). The UV lamp used in these experiments was a 365 nm UVitec lamp. As for the quantification of the concentrations of Pts, it was done by spectroscopy as explained in Section 2.3.2. Table 3 lists the different parameters and conditions tested.

2.4. Interfacial Tension Measurement. Interfacial tension was measured by the classical pendant drop method using an automated drop tensiometer (Teclis). A drop (1−20 µL) of the aqueous phase, that is suspended from a needle, is immersed in the oil phase thermostated at 30.9 °C (both for coconut and MCT oil) since the oil is less dense than the drop. All the samples were pre-equilibrated overnight prior to measurements at 30 °C. By pre-equilibration, we mean the aqueous phase and oil phase were placed in contact overnight to allow thermodynamic equilibrium before separating the two phases and carrying out the experiment.

3. RESULTS AND DISCUSSION

3.1. Effects of Pts Concentration on Emulsion Stability. Figure 1 shows the effect of the increasing concentration of Pts on coconut oil emulsions at the two storage temperatures studied. First, it should be noted that emulsions without Pts (darkest dots) are kinetically stable over 100 days. Likewise, at moderate concentrations (<38.7 g/L) of Pts, the emulsions look stable for at least 100 days. It is clear that the higher the encapsulated concentration, the more prone the emulsions are to coalescence as seen by the increase in droplet size and widening of the size distribution; however, no phase separation was observed. This effect can be observed under both storage temperatures, but at 4 °C, coalescence is kinetically delayed. The fact that at a low storage temperature of 4 °C, coconut oil is in crystalline form does not seem to affect the stability of the emulsions.

For MCT oil, which is in liquid form over all the storage temperature range, Figure 2 shows similar trends to coconut oil emulsions stored at 27 °C, where the droplet size distribution does not change in emulsions containing 0 and 36.9 g/L Pts. Droplets coalesce more rapidly at 27 °C when there is a higher concentration of Pts but still no phase separation, meaning no macroscopic oil layer at the top of the emulsion was observed. At 4 °C, however, no signs of instability are seen. It should be noted that at low temperatures, viscosities of emulsions increase, thus reducing the speed of all destabilization phenomena, which might contribute to the stability of the emulsions.[10,11]

Another noteworthy point that can be observed in Figures 1 and 2 is that with the increasing concentration of Pts, while

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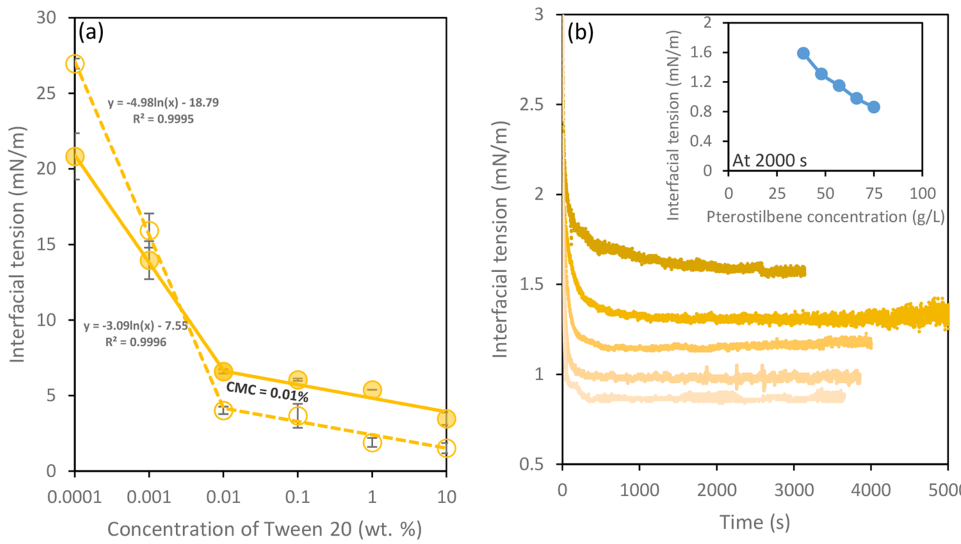

Figure 3. Interfacial tension at 30.9 °C between (a) coconut oil (filled markers) and coconut oil + 38.7 g/L Pts (empty markers) versus aqueous solutions of increasing concentration of Tween 20 and (b) coconut oil containing an increasing amount of Pts (38.7, 48.0, 57.1, 66.1, and 75.0 g/L: from lightest to darkest) and a 1% Tween 20 aqueous solution.

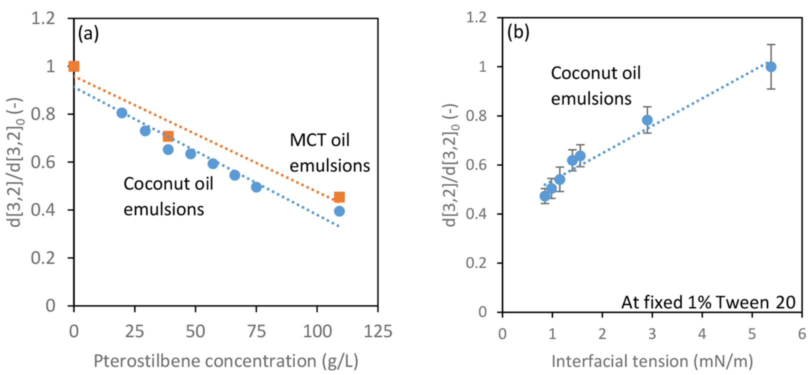

Figure 4. Sauter mean diameter homogenized by the Sauter mean diameter of the emulsion containing no Pts as a function of (a) Pts concentration and (b) interfacial tension. Lines are guides for the eyes and not a curve fitting.

maintaining the surfactant concentration constant and emulsification protocol identical, the initial oil droplet size is noticeably reduced (Figure S2 in the Supporting Information). This size reduction could be a sign of Pts surface activity, which will be discussed in the next section.

3.2. Effects of Pts Concentration on Interfacial Tension. Examining Figure 3b, it is clear that increasing the concentration of Pts in the oil phase from 38.7 to 75.0 g/L reduces, however slightly, the interfacial tension at the oil/water interface (above CMC). The fact that Pts reduces the interfacial tension leads to the conclusion that either Pts is present at the oil/water interface therefore modifying its composition: Tween 20 and Pts coexist at the interface or Pts increases the amount of Tween 20 at the interface. In both cases, Pts induces an interfacial change: an increase of adsorbed species at the interface. Moreover, Figure 4a,b shows the droplet size in the emulsions at different Pts concentrations, normalized by the droplet size in the emulsion with no Pts, as a function of Pts concentration and interfacial tension, respectively. In Figure 4a, a linear correlation exists between the reduction in droplet size and the concentration of Pts. This is because Pts concentration directly affects the interfacial tension, and Figure 4b shows that interfacial tension governs the emulsions' droplet sizes as this was already established previously.[12,13]

To further understand the effect of Pts, interfacial tension measurements were carried out between coconut oil and varying concentrations of Tween 20 (0.0001−10 wt %) as well as coconut oil containing 38.7 g/L (the threshold concentration of Pts after which emulsions became rapidly unstable) against the same Tween 20 solutions (Figure 3a). The first observation that can be made is that the critical micelle concentration point (CMC) is at 0.01 wt % Tween 20 (0.0815 mM) (with and without Pts in the oil). Note that in the Supporting Information Figure 3a is replotted with the concentration of Tween 20 in millimoles (Figure S3). From Figure 3a using the Gibbs model, we can calculate (see details in Supporting Information S4) the area covered by one Tween 20 molecule (in the absence of Pts) to be equal to 135 A^2. Under these conditions, the surface coverage is calculated to be 1.23×10^{-6} molecules m^{-2}. After the addition of Pts, Figure 3a shows a change in the slope where the surface coverage is now 1.6 times higher at 1.99×10^{-6} molecules m^{-2}. This hints at the possibility of Pts being present at the water/oil interface and modifying this interface. Although the surface coverage is higher, the interfacial tension (below CMC) is higher than that in the absence of Pts. This might be due to an interaction occurring between Pts and the fatty acids in coconut oil. Looking at the interfacial tension values at 0.0001% Tween 20 (that is similar to water), adding Pts to the oil

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increased the interfacial tension from 21 to 27 mN/m. The literature shows that interfacial tension increases with the increasing fatty acids chain length.[14,15] In this sense, we hypothesize that Pts is bonding to the small fatty acid chains in coconut oil, rendering the oil more hydrophobic, thus increasing the interfacial tension; however, proving this remains out of the scope of this study. What is noteworthy, however, is plotting ΔIT versus Pts concentration for both coconut and MCT oil, where ΔIT is calculated using eq 3.

$$\Delta\ \mathrm{IT} = \delta_{\mathrm{water/oil+PTS}} - \delta_{\mathrm{water/oil}} \quad (3)$$

where δ is the equilibrium interfacial tension. Figure 5 shows that Pts in MCT oil has a much more significant effect on interfacial tension than in coconut oil, and this might be because MCT oil contains shorter triglyceride chains than coconut oil, thus rendering MCT oil more hydrophobic. MCT oil, being composed of C8 and C10 carbon chains, has a higher concentration of these medium-sized chains compared to coconut oil, which is made up mostly of C12 and C14 carbon chains.[16] Dervichian[17] reports that longer fatty acid chains increase the cohesion energy in the oil, which in turn increases interfacial tension. Nevertheless, this Pts−fatty acid interaction seems to be above CMC where the presence of Pts lowers the interfacial tension twofold. This is due to the higher surface coverage as discussed earlier.

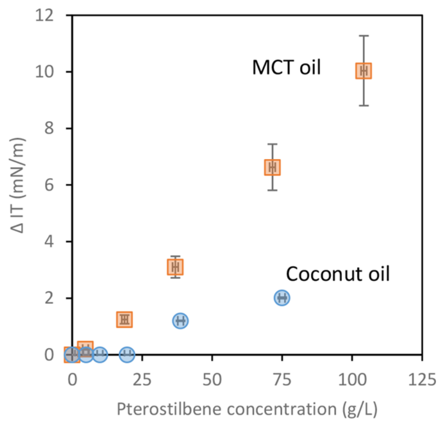

Figure 5. ΔIT versus different concentrations of Pts (0−75 g/L) in coconut oil and (0−104.2 g/L) in MCT oil.

In the presence of Tween 20, similar interfacial tension trends are obtained with MCT oil (Figure S5 in the Supporting Information). It should be noted that Pts is soluble in Tween 20 and the increase in interfacial tension (below CMC) could also be due to a part of the Tween 20 molecules being no longer available to stabilize the oil/water interface since they are bonding with Pts molecules that have moved from the oil phase to the aqueous phase. In fact, preparing a sample of 1% Tween 20 and 0.82 g/L Pts and using a dynamic light scattering equipment, a micelle peak (d: 8.0 nm; PDI: 0.0094) was observed (Figure S6 in the Supporting Information). This is the size of Tween 20 micelles reported in the literature, which means that Pts is dissolved and is not forming micelles.[18] Accordingly, above CMC, Pts could partition between the aqueous continuous phase containing Tween 20 micelles and the oil-dispersed phase. Consequently, it is important to measure the encapsulation efficiency (proportion of Pts inside the drops) of the emulsions (Section 3.3).

3.3. Encapsulation Efficiency. On a macroscopic scale and to quantify, in the produced emulsions, how much Pts passes from the oil to the aqueous phase (encapsulation efficiency), a spectroscopy approach was taken. Coconut oil/Pts solutions were prepared at the theoretical values of 38.7 (where Pts is completely solubilized) and 109.1 g/L (beyond the solubility

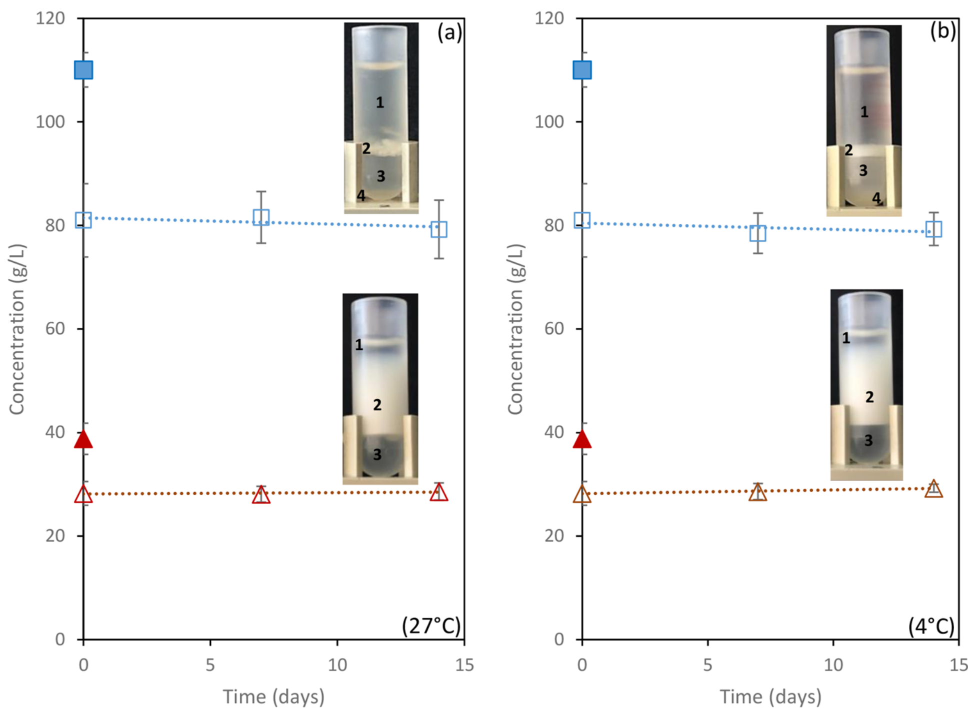

Figure 6. Concentration of Pts in 70% coconut oil emulsions stored at (a) 27 °C and (b) 4 °C. Empty markers: Pts concentration in oil after emulsification: triangles: 38.7 g/L and squares: 109.1 g/L. Filled markers: concentration in oil before emulsification. The phases in the images are: (1) oil, (2) emulsion, (3) aqueous phase, and (4) gel. Lines are guides for the eyes and not a curve fitting.

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limit at 30 °C and where part of Pts remains suspended in the oil), and MCT oil/Pts solutions were prepared at the theoretical values of 36.9 and 104.2 g/L (where Pts is completely solubilized at both concentrations). 70 wt % oil-in-water emulsions were then prepared. Using the standard curve described in (Section 2.3.2) and eq 2, it was possible to calculate the unknown concentrations of Pts in the oil phases.

The real concentrations of Pts were measured before emulsification (Figure 6), and then, once the emulsion was prepared, it was ruptured by centrifugation, and the oil on top was used to measure the concentration of Pts that was encapsulated. Figure S7 (Supporting Information) shows that the determined values of the Pts concentrations in the oil before emulsification are in agreement with the theoretical ones for the several concentrations tested out. Moreover, Figure 6 shows that a part of Pts has passed to the aqueous phase in Tween 20-stabilized coconut oil emulsions. Saying this, and knowing that Pts is hydrophobic, signifies that the presence of Tween 20 has increased the solubility of Pts in the aqueous phase. Figure 7 shows that 26 and 16% of Pts in the coconut oil and MCT oil emulsions, respectively, have moved to combine with the nonadsorbed Tween 20 molecules in the aqueous phase. Taking a closer look at the centrifuged samples in Figure 6, one can see four phases at high Pts concentration and three phases at low Pts concentrations. From top to bottom the four phases are (1) oil, (2) emulsion, (3) aqueous, and (4) gel-like phases. This latter phase is not present at low Pts concentrations. Thus, the observed gel-like phase in the images of the centrifuged emulsions containing 109.1 g/L Pts might possibly lead to the instability that was observed in the concentrated emulsions in Figure 1. Also, it should be noted that since the emulsion containing 109.1 g/L is less stable, under the same centrifugal forces, the phases of this emulsion completely separated as compared to the emulsion containing 38.7 g/L where barely any oil phased off (images in Figure 6).

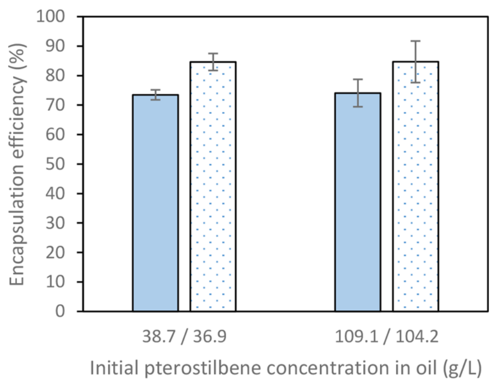

Figure 7. Percentage of Pts that passed from the oil to the aqueous phase in coconut (solid columns) and MCT oil (dotted columns) emulsions.

The higher encapsulation efficiency in MCT oil emulsions might be due to Pts being more soluble in MCT oil than in coconut oil;[7] not to mention that at a high concentration of Pts of 109.1 g/L in coconut oil, Pts molecules remain suspended in the oil rather than dissolved (solubility limit surpassed). Furthermore, we observe in Figure 6 that the concentration of Pts that has passed to the aqueous phase remains stable over time, which means that this transfer was done at the moment of emulsification and no further transfer happens afterward, at least for the 2 week study period. The same observation can be made for MCT oil emulsions (results in Figure S8 in the Supporting Information). In the literature, the encapsulation efficiency of MCT oil nanoemulsions encapsulating Pts and stabilized by soybean lecithin was measured and reported to be about 100% over a period of 28 days.[7] Unlike Tween 20, lecithin does not allow the transfer of Pts from the oil to the aqueous phase, which explains the difference in encapsulation efficiency.

3.4. Effect of UV Light on the Stability of Pts. With the aim of testing the stability of Pts to light, a preliminary test was done in ethanol at 0.55 g/L Pts. One sample was placed in the dark as a control and another under a UV lamp. As shown in Figure 8a, about 75% of the Pts dissolved in ethanol has degraded in the samples stored under UV light (in 18 min), while no change was observed in the control sample stored in the dark. This is in line with the significant degradation of Pts due to light, reported in the literature.[19,20] With the aim of improving Pts stability, the effect of adding vitamin E was tested. Two samples of ethanol containing 0.55 g/L Pts, one with vitamin E (0.015 wt %) and the other without, were placed under a UV lamp for 1 h. After 1 h, the sample containing no vitamin E lost 75% of its Pts content, whereas the other one maintained 100% of the Pts molecules (Figure 8b).

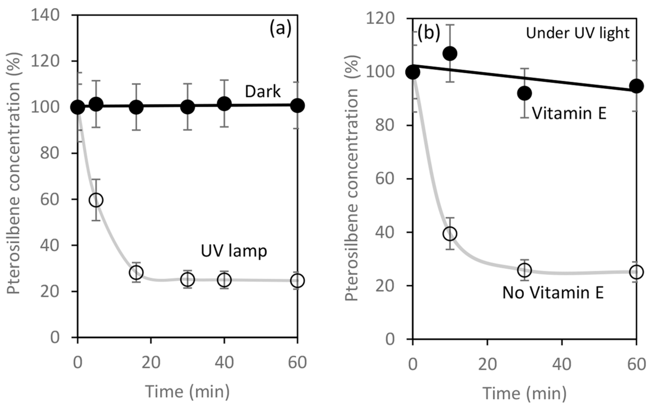

Figure 8. Pts at 0.55 g/L in ethanol under (a) different light conditions and (b) in the presence or absence of vitamin E. Lines are guides for the eyes and not a curve fitting.

It was desirable to observe how the stability of Pts would be affected when encapsulated in an emulsion. Emulsions without vitamin E were first produced using coconut and MCT oil and stored under UV light at 4 and 27 °C (Table 3). It is clear that after 7 days under UV light, the emulsions (regardless of the oil type or storage temperature) were able to maintain almost 100% of the encapsulated Pts (Figure 9a). Unlike the transparent ethanol solution, emulsions have a white color due to multiple light scattering. Therefore, the light is deviated and is not allowed to travel deep into the sample, thus protecting the encapsulated active ingredient. However, when the oil containing Pts was not encapsulated in an emulsion, Pts concentrations decreased with time. Comparing coconut oil containing Pts stored at 4 and 27 °C under a UV light, it is clear that at 4 °C, where coconut oil is in its crystalline form, the percentage of Pts lost (22%) was less than that in liquid coconut oil (40%). Again, this is due to the ability of crystals to diffuse light. Similar results to liquid coconut oil were obtained with MCT oil where 45% of Pts was degraded.

In the experiments with 10 wt % vitamin E added to the oil phase (Figure 9b), the coconut emulsion showed no difference from that without the antioxidant, and the protection comes

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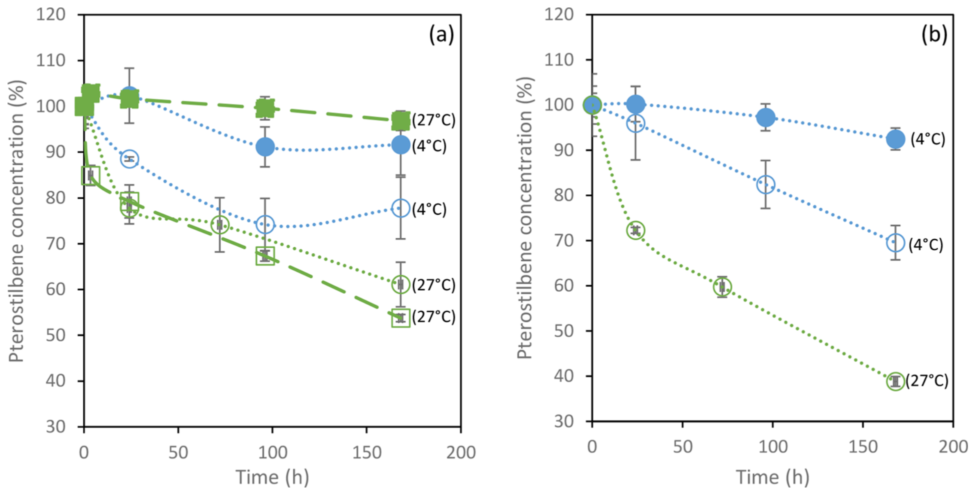

Figure 9. Concentration of Pts over time in 70% coconut oil emulsions containing coconut oil (circles) or MCT oil (squares). Filled markers refer to emulsions and empty markers refer to nonencapsulated oil. Samples were prepared (a) without and (b) with 10 wt % vitamin E. Lines are guides for the eyes and not a curve fitting.

mainly from light diffusion. Coconut oil at 4 °C with vitamin E also showed a similar reduction (70%) of Pts concentration as that with no vitamin E due to the white crystalline form of the oil. Finally, the coconut oil with vitamin E in the liquid form lost 61% of Pts, which was not expected especially when compared with the results seen in ethanol (Figure 8b). However, a review of the literature showed that the concentration of an antioxidant is quite sensitive and a large amount will catalyze the oxidation reaction,[21] which is likely what happened in this case.

4. CONCLUSIONS

Pts is known to be hydrophobic and difficult to encapsulate. In this study, it was possible to encapsulate this molecule up to 66.9 g/L (with respect to the total emulsion) in concentrated, Tween 20-stabilized, oil-in-water emulsions (70 wt % of oil). Coconut emulsions were more sensitive to the storage temperature in terms of emulsion stability compared to MCT emulsions. In the presence of Tween 20, Pts passes to the aqueous phase, causing emulsions containing very high concentrations of Pts (>100 g/L) to destabilize quicker due to a gel formation in the aqueous phase. Measuring the concentration of Pts that has passed to the aqueous phase, a 23−30% transfer occurred in coconut oil emulsions, which is higher than that occurring in MCT oil emulsions (16% or 12.7 g/L of the total emulsion). Finally, the most promising emulsions are MCT emulsions with 79.6 g/L Pts (in the total sample) and stored at 4 °C. The use of emulsions, that diffuse light, is also very efficient in preserving Pts against light degradation, and no addition of antioxidants is required.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c01861.

> Interfacial tension measurements between MCT oil and water/Tween 20 solutions, the encapsulation efficiency of MCT oil emulsions, the spectroscopy calibration curve, and the measured vs theoretical Pts concentration curve; optical microscopy pictures of coconut oil emulsions stabilized with Tween 20 with different concentrations of Pts; plots of Tween 20 micelle size; and the calculations of Tween 20 surface concentration (PDF)

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Notes

The authors declare no competing financial interest.

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