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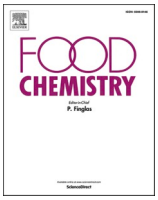

Development and characterization of nanostructured lipid carriers for cannabidiol delivery

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ABSTRACT

This study evaluated the physicochemical characteristics of nanostructured lipid carriers (NLCs) as a potential vehicle for cannabidiol (CBD), a lipophilic molecule with great potential to promote health benefits. NLCs were produced using hemp seed oil and fully-hydrogenated soybean oil at different proportions. The emulsifiers evaluated were soybean lecithin (SL), Tween 80 (T80) and a mixture of SL:T80 (50:50). CBD was tested in the form of CBD-rich extract or isolate CBD, to verify if it affects the NLCs characteristics. Based on particle size and polydispersity, SL was considered the most suitable emulsifier to produce the NLCs. All lipid proportions evaluated had no remarkable effect on the physicochemical characteristics of NLCs, resulting in CBD–loaded NLCs with particle size below 250 nm, high CBD entrapment efficiency and CBD retention rate of 100% for 30 days, demonstrating that NLCs are a suitable vehicle for both CBD-rich extract or isolate CBD.

1. Introduction

The recent legalization of cannabis and its products in many countries around the world has opened an extraordinary opportunity for the creation of a new industry, especially in North America and Europe, which has created a veritable race to develop new cannabis-based products (Marangoni & Marangoni, 2019). Cannabis has been promoted from the commonly used recreational drug to a promising natural drug for many health and well-being interests, which has been boosted the development of cannabis edibles, one of the greatest opportunities to take place in this fast-growing market (de Aguiar et al., 2023).

Cannabidiol (CBD) is one of the most abundant bioactive compounds found in cannabis and has been extensively studied for the treatment of several diseases, including epilepsy, cancer, inflammatory diseases, and many other disorders (Abrams, 2018). However, to be placed on the European market, CBD oils, supplements, food and drink products require a premarket Novel Food authorization to assure the safety of these products (EU-Regulation 2015/2283).

CBD presents a poor oral bioavailability owing to the CBD lipophilicity and marked liver first-pass metabolism, which represent two of the most important challenges that need to be addressed in the development of cannabis-based products (Perucca & Bialer, 2020). In addition, the poor water solubility of CBD also represents a challenge to its incorporation into water-based edible products (Franco et al., 2020). Thus, the incorporation of CBD into lipid-based delivery systems, such as food grade nanostructured lipid carriers (NLC) can potentially provide a solution to enhance the oral bioavailability of CBD and enable its incorporation in a broader range of water-based edible products (Franco et al., 2020; Perucca & Bialer, 2020; Zielińska et al., 2023). NLCs are a type of lipid-based nanocarriers composed of a mixture of solid and liquid lipids dispersed in aqueous medium stabilized by a emulsifier (Gonçalves et al., 2018; Lüdtke et al., 2022). In addition of being claimed for improving the bioavailability of lipophilic bioactive compounds, NLCs also present the advantages of high encapsulation efficiency, loading capacity, physical stability, control release, biodegradability, and low toxicity (Gonçalves et al., 2018). Furthermore, their easy dispersion in aqueous medium, nanoscale size, and biocompatibility enhances the ability of NLCs to deliver lipophilic molecules in aqueous-based food products such as beverages, candies, gummies, among others (McClements, 2020). Another aspect to be considered when designing cannabis edibles is the potential of synergistic effects between the different types of cannabinoids, as well as, with non-cannabinoid components, such as drugs, nutraceuticals, and food ingredients (McClements, 2020). Studies have reported that CBD-

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rich extracts present greater activity compared to single molecules, which enables reducing the administered doses due to the entourage effect (Pamplona et al., 2018; Russo, 2019).

The incorporation of CBD into lipid-based delivery systems has already been investigated. Nanoemulsions (NE) containing CBD-rich extracts were successfully obtained with droplet size below 100 nm when synthetic surfactants (Tween 80/Span 80 mixture) were used but not with the natural surfactant Q-naturale® (Leibtag & Peshkovsky, 2020). On another study, NE containing CBD distillate was obtained using Q-natulare® as surfactant resulting in particles with average droplet size of 120 nm, which demonstrated to be stable to droplet agglomeration and phase separation for 6 weeks (Banerjee et al., 2021). The findings of these studies suggest that depending on the form that the CBD is used (CBD-rich extract or CBD distillate), its interaction with the surfactant in the nanostructure can be affected. Mucoadhesive NLCs containing CBD for the treatment of neuropatic pain were developed by Matarazzo et al., (2021) which obtained particles with size below 200 nm and entrapment efficiency of 99.99%. A self-emulsifying drug delivery system (SEDDS) was also developed to carry a CBD-rich extract, which presented a mean particle size of 148 nm (De Prá et al., 2021), while self-nanoemulsifying drug delivery system (SNEEDS) containing isolate CBD resulted in particles with droplet size below 50 nm (Izgelov et al., 2020).

In spite of the interesting results, these works mainly focus on pharmaceutical and nutraceutical applications. Therefore, the delivery systems for CBD are generally obtained using purified components, which means that, although they are acceptable for oral ingestion, in most cases they are not economically feasible for food applications due to the elevated costs of the purified constituents. In this regard, the development of NLCs based on food grade ingredients is of great interest to promote its application in foods.

Fully hydrogenated soybean oil (FHSO) is a vegetable oil with high melting point that has been used as an alternative to replace the purified fatty acids (for example, stearic acid) generally applied to obtain NLCs, aiming at reducing the production costs (Lüdtke et al., 2022; Santos et al., 2019). Hemp seed oil (HSO) presents a chemical profile mainly composed of long-chain triglycerides, which were claimed to increase the bioavailability of lipophilic molecules in comparison with short- and medium-chain triglycerides (De Prá et al., 2021), because they divert the cannabinoids absorption from the portal vein to the lymphatic system, thus bypassing the liver and decreasing the pre-systemic metabolism (Izgelov et al., 2020). To the best of our knowledge, HSO has not been used to produce NLCs yet, however, HSO nanoemulsions were successfully produced to carry supplements and bioactive compounds such as iron, vitamin D (Smułek & Jarzębski, 2023), and ascorbic palmitate (Amiri-Rigi et al., 2023).

The type of emulsifier used to obtain NLCs can also influence their characteristics (Lüdtke et al., 2022). Derived from polyethoxylated sorbitan and oleic acid, Tween 80 is a nonionic emulsifier considered suitable to stabilize NLC due to their hydrophilic/lipophilic balance (HLB) (~15) (Mohammadi et al., 2019). On the other hand, soybean lecithin (SOLEC™ AE IP) (HLB 7–8) is an enzymatically hydrolyzed lecithin which presents a great emulsifying capacity for stabilization of oil/water-type emulsified systems due to their high lysophospholipid/phospholipid ratio (Fernandes et al., 2012), and meets the current appeal for the use of natural plant-based ingredients (Katouzian et al., 2017).

Thus, NLCs formulated with different emulsifiers and lipidic phase compositions were developed to carry CBD-rich extracts and isolate CBD aiming at evaluating its effects on the physical–chemical properties, entrapment efficiency, and stability of the NLCs, in view of subsequent applications as CBD-carrier systems with improved bioavailability.

2. Material and methods

2.1. Materials

To produce the NLCs, hemp seed oil (HSO, Natursoy, Barcelona, Spain) was used as the liquid lipid and fully hydrogenated soybean oil (FHSO, Cargill Foods, Sao Paulo, Brazil) was used as solid lipid. Enzymatically hydrolyzed soybean lecithin (SL) (SOLEC™, Solae, Esteio, Brazil) and Tween® 80 (T80) (Sigma-Aldrich, Saint Louis, USA) were used as emulsifiers. Isolate cannabidiol (CBD_{iso}, 99.2%) and Broad-spectrum CBD distillate (CBD_{ext}, 87.0%) were gently donated by Essentia Pura (Ljubljana, Slovenia). Acetonitrile (HPLC grade, $\geq$ 99.9%,) was provided by Fischer Chemical (Hampton, USA) and formic acid (99–100%) was provided by Chem-Lab (Zedelgem, Belgium). The analytical standard CBD (Cerilliant®, 1.0 mg/ml in methanol) was provided by Sigma-Aldrich (Saint Louis, USA).

2.2. NLC preparation

Firstly, unloaded-NLCs containing different proportions of emulsifiers (T80:SL 100:0, 50:50 and 0:100 w/w) were evaluated to select the most suitable emulsifier to further produce the loaded-NLCs (Table S1). For this purpose, the NLCs were produced through pre-emulsification followed by ultrasonication as described by Gonçalves et al. (2021). A pre-emulsion constituted by the lipid phase (10%) (HSO:FHSO 50:50, w/w), the emulsifier (3%), and ultrapure water (87%) was prepared by solubilizing the SL in the lipid phase, while T80 was solubilized in the aqueous phase. The aqueous phase was heated at 85 °C, quickly dispersed in the lipid phase, and homogenized with an Ultra-Turrax (T18, Ika-Werke, Staufen, Germany) at 7,000 rpm for 5 min. The pre-emulsion was then subjected to high-intensity ultrasonication using a titanium microtip (3.0 mm diameter, 20 kHz, Vibra-cell VCX 500, Sonics & Materials, Newtown, USA) with an amplitude of 40% for 4 min with pulses (4 s on; 2 s off) at 75 °C. Finally, the NLCs were dispersed in cold water (2 °C) at a ratio of 1:10 under stirring at 600 rpm for 15 min (Gonçalves et al., 2021). The NLCs were produced in triplicate and stored in amber glass flasks at ambient conditions (23 ± 2 °C) for 30 days.

The most suitable emulsifier was selected based on the mean particle size (PS), polydispersity index (PDI) and stability. So, CBD_{iso} and CBD_{ext} loaded-NLCs and unloaded-NLCs using different proportions of the lipid phase (HSO:FHSO 50:50, 60:40 and 70:30 w/w) and SL as emulsifier were produced according to the same conditions previously described (Table S1). For the loaded-NLCs, the active compound (CBD_{iso} or CBD_{ext}) at concentration of 1% was also melted in the lipid phase. The NLCs were produced in triplicate and stored in amber glass flasks at ambient conditions (23 ± 2 °C) for 30 days.

2.3. NLC characterization

2.3.1. Particle size (PS) and polydispersity index (PDI)

The mean PS and PDI of the NLCs were measured in triplicate at 25 °C using dynamic light scattering (Zetasizer Nano SZ, Malvern, Worcestershire, UK). Prior to the analysis, the samples were diluted at 1:100 with ultrapure water. The oil refractive index and particle absorbance values used in Malvern software were 1.47 and 0.001, respectively. The PS and PDI were evaluated at 24 h, 14, 21 and 30 days for unloaded-NLCs produced with different proportions of emulsifiers, and at 24 h, 7, 14, 21 and 30 days for loaded- and unloaded-NLCs produced with different proportions of lipid phase. The mean PS was expressed as z-average (nm).

2.3.2. Differential scanning calorimetry (DSC)

The thermal behavior of the NLCs was evaluated in a differential scanning calorimeter (DSC 6000, Perkin Elmer, Waltham, USA) according to the conditions previously described by Lüdtke et al. (2022).

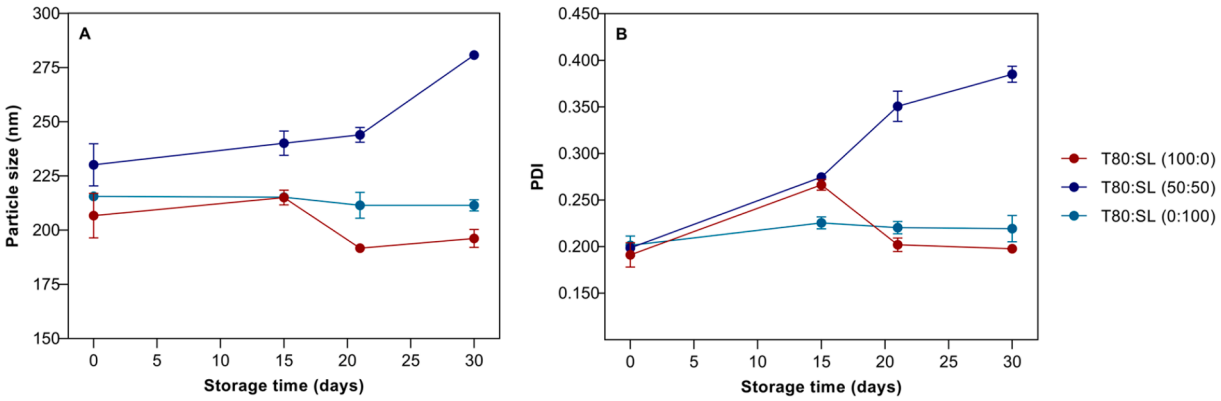

Fig. 1. Particle size expressed as z-average diameter (A) and polydispersity index (B) of the NLC obtained with different emulsifiers. T80: Tween 80®; SL: Soybean lecithin.

Around 10 mg of sample were weighed in triplicate and sealed into pinholder aluminum pans. An isotherm was hold at 25 °C for 10 min, followed by temperature increase from 25 °C to 95 °C at a fixed rate of 10 °C/min and pure nitrogen gas flow of 20 mL/min. The thermal behavior was evaluated in triplicate at 0, 15, and 30 days of storage.

2.3.3. X-ray diffraction (XRD)

The polymorphic form of NLCs was analyzed by XRD according to the AOCS Cj 2–95 (AOCS, 2009) using a XRD equipment (Bruker D8, Odelzhausen, Germany) with Bragg-Bretano geometry (θ:2θ) with Cu-Kα radiation (λ = 1.54056 Å, 40 kV, 30 mA). The measures were obtained with 0.02° steps from 5° to 40° (2θ) and acquisition time of 2 s. To prepare the samples, 1 mL of NLCs was placed on a glass blade and left over night to evaporate the water at room temperature.

2.3.4. Fourier-transform infrared spectroscopy (FTIR)

The FTIR analysis was performed in a Fourier-transformed infrared spectrometer (Bruker, Alpha II, Billerica, USA) equipped with attenuated total reflectance (ATR). The NLCs samples were directly analyzed with a spectral resolution of 4 cm^{-1}, 48 scans in the range of 375 – 4000 cm^{-1}.

2.3.5. Entrapment efficiency

The entrapment efficiency (EE) was indirectly determined in triplicate by the ultrafiltration/centrifugation method using Amicon® ultrafiltration devices (100 kDa cut-off, Millipore, Burlington, USA) according to described by Matarazzo et al. (2021) with some modifications. 8 μL of the NLC dispersions were diluted in 495 μL of ultrapure water, added to the upper part of the Amicon® device and centrifugated at 4,000 rpm for 60 min (Mikro 120, Hettich Centrifuge, Tuttlingen, Germany). The amount of CBD in the filtered (free CBD) phase was determined by Ultra-High Performance Liquid Chromatography (UHPLC) coupled to a diode array detector (Nexera X2, Shimadzu, Kyoto, Japan) according to the method described by De Prá et al. (2021). A C18 column (Kinetex, 150 mm × 2.6 mm, 2.1 μm, Phenomenex, Torrance, USA) was used for the compounds' separation. The mobile phased was constituted of water (A) and acetonitrile (B), both acidified with 0.1% formic acid in a gradient elution mode at a flowrate of 0.5 mL/min as follows: 0 min, 34% A; 8 min, 34% A; 12 min, 5% A; 13 min, 5% A; 17 min, 34% A; 18 min, 34% A. The column temperature was maintained at 30 °C and data acquisition was performed at 220 nm. The CBD was identified and quantified using an external calibration curve of CBD in the range of 1 and 50 μg/mL (r^2 = 0.998) The EE was calculated using Eq. (1):

$$EE(\%) = \left(\frac{TotalCBD - FreeCBD}{TotalCBD}\right) \times 100 \quad (1)$$

2.4. Statistical analysis

The results were presented as mean ± standard deviation (n = 9). Data were analyzed using one-way analysis of variance (ANOVA) and Tukey's test to evaluate significant differences between the mean values at a confidence level of 95 % (p-value ≤ 0.05) using Minitab 20.0 software (State College, USA).

3. Results and discussion

3.1. Emulsifier selection

As the type and ratio between the solid and liquid lipids and the type of emulsifier used to obtain the NLCs typically influence their physicochemical characteristics (Badea et al., 2015; How et al., 2013; Lüdtke et al., 2022), the type of emulsifier and different ratios between FHSO and HSO were evaluated in the present study. The emulsifiers evaluated to obtain the NLCs were the SL, T80, and an equal ratio between SL:T80 (50:50, w/w). SL is a natural emulsifier mainly composed of lysophospholipids (Fernandes et al., 2012), while T80 is a commonly used synthetic nonionic emulsifier, derived from polyethoxylated sorbitan and oleic acid (Mohammadi et al., 2019). The selection of the most suitable emulsifier was based on the PS, PDI, and stability of the NLCs. The PS and PDI of the NLCs obtained with different emulsifiers over storage time are presented in Fig. 1. It can be seen that all emulsifiers resulted in NLCs at nanometric scale but presented significantly different behaviors during the storage time (Table S2). The NLC obtained with T80 as emulsifier (T80:SL (100:0)) presented the smallest PS in the range of 191.7 – 215.0 nm. Similar behavior was observed by other authors for NLCs obtained with high oleic sunflower oil (HOSO) and FHSO at different ratios ranging from HOSO:FHSO (20:80) to HOSO:FHSO (80:20) using 2% of T80 as emulsifier that resulted in PS (d_{32}) between 160.1 and 223.0 nm (Lüdtke et al., 2022). Emulsifiers with high ability to reduce the surface tension positively impact the formation of NLCs by reducing their particle size (Håkansson et al., 2013). T80 is largely used as emulsifier due to its low molecular weight that enables a quick adsorption at the oil–water surface, resulting in NLCs with small PS, as observed in the present study.

The NLCs obtained with SL (T80:SL (0:100)) presented PS slightly higher than the ones obtained with T80 (in the range of 211.5 – 219.2 nm) and no significant differences were observed during the storage time of 30 days (Table S2). These results can be associated with the structural and chemical similarity between SL and triacyclglycerols (TAG), which makes their association easier, thus resulting in NLCs with low PS (Lüdtke et al., 2022). In contrast, the NLC obtained with both emulsifiers, T80:SL (50:50) presented the largest PS, from 230.1 nm at the day 0 (just after the preparation) reaching 280.7 nm after 30 days of storage. These largest PS probably occurred due to structural differences

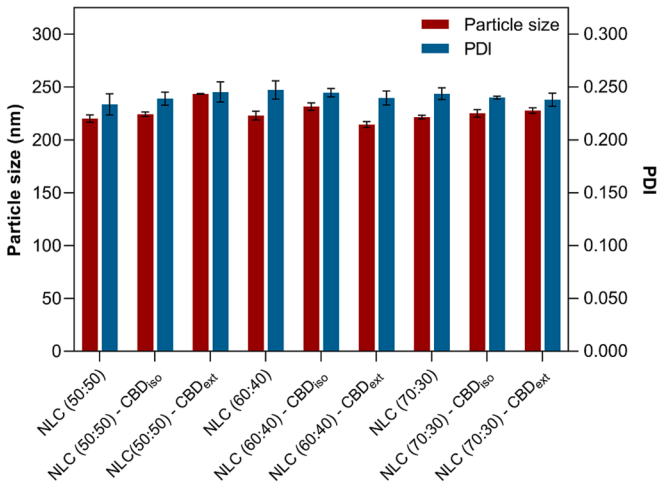

Fig. 2. Particle size (nm) expressed as z-average diameter and polydispersity index of loaded and unloaded NLC composed of different HSO:FHSO ratios. HSO: hemp seed oil; FHSO: fully hydrogenated soybean oil; CBD_{iso}: Isolate cannabidiol; CBD_{ext}: Broad-spectrum CBD distillate.

between T80 (non-ionic) and SL (zwitterionic), which may have led to less drop coverage during the NLC production process, and consequently to higher PS than those obtained with SL and T80 individually. Gonçalves et al. (2021) also used SL (Phospholipon® 90 G) and T80 (50:50) to produce NLCs and observed a particle diameter of 164.4 ± 10.3 nm. The lower PS observed by these authors may be related to the lipidic composition of the NLCs, since the authors used medium-chain triglycerides (MCTs) and beeswax as liquid and solid lipids, respectively, which present lower molecular weight than the lipids used in the present study.

Regarding the PDI, all NLC presented values below 0.200 at the day 0 (Fig. 1b) with no significant difference between them (Table S2). However, during the storage time, the PDI of the NLCs obtained with only T80 or SL remained practically constant, while the NLC obtained with T80:SL (50:50) presented a significant increase in the PDI reaching a value of 0.385. The PDI values found for NLC obtained with T80:SL (50:50) were consistent with the increase in PS during storage and indicate that the use of emulsifiers individually was more effective to produce stable NLCs with smaller and narrower sizes. Based on these results, and considering the constant search for replacing synthetic ingredients with natural ones, SL was selected as emulsifier for the further steps of this study because it enabled obtaining stable NLC with low PS.

3.2. Effect of lipidic phase composition

3.2.1. Particles characterization

3.2.1.1. Particle size and polydispersity index. The effect of lipidic phase composition was evaluated on unloaded NLCs obtained with HSO:FHSO ratios of 50:50, 60:40, and 70:30 (w/w), and on the same NLCs loaded with isolate CBD (CBD_{iso}) and broad-spectrum CBD distillate (CBD_{ext}) to observe if the presence of CBD_{iso} and CBD_{ext} into nanostructures differently affects their properties. Fig. 2 presents the PS and PDI of the NLCs. The PS of the unloaded NLCs were approximately 220 nm with PDI values around 0.250, indicating a narrow size distribution (PDI < 0.300) and characterizing the NLCs as suitable for oral delivery (Gonçalves et al., 2021). The different proportions of liquid and solid lipids presented no significant differences on the values of PS and PDI. Other authors observed that NLCs obtained by high pressure homogenization using similar components constituting 10% of lipid phase (FHSO and high oleic sunflower oil, HOSO, at different proportions) and 2% of SL as emulsifier presented PS in a similar range (216–255 nm) of the present study but, in that case, the authors observed a significant decrease on the

Table 1
Particle size and PDI of unloaded and loaded NLC's obtained with different lipidic compositions evaluated during 30 days of storage.

	Particle size (nm)				
NLC	24 h	7 days	15 days	21 days	30 days
HSO:FHSO (50:50)	220.2 ± 3.3 ^{a}	220.5 ± 2.9 ^{a}	226.3 ± 1.8 ^{a}	219.6 ± 2.7 ^{a}	218.7 ± 3.9 ^{a}
HSO:FHSO (50:50) – CBD_{iso}	224.3 ± 2.2 ^{ab}	224.7 ± 3.4 ^{ab}	226.7 ± 0.2 ^{a}	222.0 ± 2.1 ^{ab}	219.1 ± 0.8^{b}
HSO:FHSO (50:50) – CBD_{ext}	243.5 ± 0.4 ^{a}	219.5 ± 1.4^{b}	219.6 ± 3.2^{b}	216.7 ± 3.3 ^{bc}	212.6 ± 2.7^{c}
HSO:FHSO (60:40)	222.9 ± 4.2 ^{a}	222.2 ± 4.3 ^{a}	225.7 ± 2.9 ^{a}	218.2 ± 2.6 ^{a}	218.4 ± 1.6 ^{a}
HSO:FHSO (60:40) – CBD_{iso}	231.5 ± 3.5 ^{a}	227.0 ± 3.2 ^{ab}	218.4 ± 0.1^{c}	224.1 ± 1.8 ^{abc}	222.5 ± 4.3 ^{bc}
HSO:FHSO (60:40) – CBD_{ext}	214.6 ± 2.9 ^{a}	214.8 ± 1.2 ^{a}	214.1 ± 0.4 ^{a}	212.0 ± 2.6 ^{ab}	207.7 ± 1.2^{b}
HSO:FHSO (70:30)	221.6 ± 1.8 ^{ab}	217.0 ± 2.6^{b}	223.6 ± 2.9 ^{a}	221.33 ± 3.1 ^{ab}	215.8 ± 0.9^{b}
HSO:FHSO (70:30) – CBD_{iso}	225.1 ± 3.5 ^{a}	219.1 ± 2.4 ^{ab}	220.8 ± 4.3 ^{ab}	219.07 ± 1.8 ^{ab}	215.8 ± 3.2^{b}
HSO:FHSO (70:30) – CBD_{ext}	227.9 ± 2.6 ^{a}	224.1 ± 1.8 ^{a}	225.9 ± 1.6 ^{a}	223.80 ± 2.1 ^{a}	227.1 ± 3.0 ^{a}
	PDI				
NLC	**24 h**	**7 days**	**15 days**	**21 days**	**30 days**
HSO:FHSO (50:50)	0.234 ± 0.010^{b}	0.250 ± 0.007 ^{ab}	0.266 ± 0.008 ^{a}	0.254 ± 0.003 ^{a}	0.246 ± 0.008 ^{ab}
HSO:FHSO (50:50) – CBD_{iso}	0.239 ± 0.006 ^{a}	0.239 ± 0.016 ^{a}	0.262 ± 0.012 ^{a}	0.244 ± 0.017 ^{a}	0.243 ± 0.008 ^{a}
HSO:FHSO (50:50) – CBD_{ext}	0.245 ± 0.009 ^{a}	0.234 ± 0.008 ^{a}	0.253 ± 0.013 ^{a}	0.238 ± 0.017 ^{a}	0.248 ± 0.007 ^{a}
HSO:FHSO (60:40)	0.247 ± 0.009^{b}	0.227 ± 0.011^{b}	0.296 ± 0.028 ^{a}	0.257 ± 0.024 ^{ab}	0.248 ± 0.005^{b}
HSO:FHSO (60:40) – CBD_{iso}	0.245 ± 0.004 ^{ab}	0.249 ± 0.008 ^{ab}	0.240 ± 0.01 ^{ab}	0.255 ± 0.003 ^{a}	0.238 ± 0.001^{b}
HSO:FHSO (60:40) – CBD_{ext}	0.240 ± 0.007 ^{a}	0.221 ± 0.027 ^{a}	0.239 ± 0.005 ^{a}	0.244 ± 0.007 ^{a}	0.222 ± 0.001 ^{a}
HSO:FHSO (70:30)	0.244 ± 0.001 ^{ab}	0.228 ± 0.007^{b}	0.267 ± 0.009 ^{a}	0.269 ± 0.024 ^{a}	0.239 ± 0.003 ^{ab}
HSO:FHSO (70:30) – CBD_{iso}	0.240 ± 0.001 ^{a}	0.243 ± 0.006 ^{a}	0.270 ± 0.015 ^{a}	0.252 ± 0.007 ^{a}	0.241 ± 0.018 ^{a}
HSO:FHSO (70:30) – CBD_{ext}	0.238 ± 0.006 ^{a}	0.250 ± 0.010 ^{a}	0.251 ± 0.011 ^{a}	0.253 ± 0.012 ^{a}	0.246 ± 0.010 ^{a}

Average of three replicates ± Standard $^{a\text{-}c}$ different letters in the same line represent significant difference (p-value ≤ 0.05) during the storage for the same NLC.

PS with the increase of FHSO content (Lüdtke et al., 2022). Tamjidi et al. (2014) reported that the addition of liquid lipids in the NLCs production can increase the PS due to interfacial tension increase, nanoparticles swollen core increase or core–shell type nanoparticles production. In this study, different behaviors were observed for the CBD_{iso} and CBD_{ext}-loaded NLCs produced with different lipid compositions, which can be attributed to the interactions between each form of CBD and the constituents of the NLCs.

The loading of CBD_{ext} into the nanostructure resulted in an increase of PS only in NLC composed by HSO:FHSO (50:50), indicating a lower interaction of the extract with the solid lipid (FHSO). On the other hand, PS results indicate that the increase of the liquid lipid in the NLC composition facilitate the CBD_{ext} solubilization into the structure, since

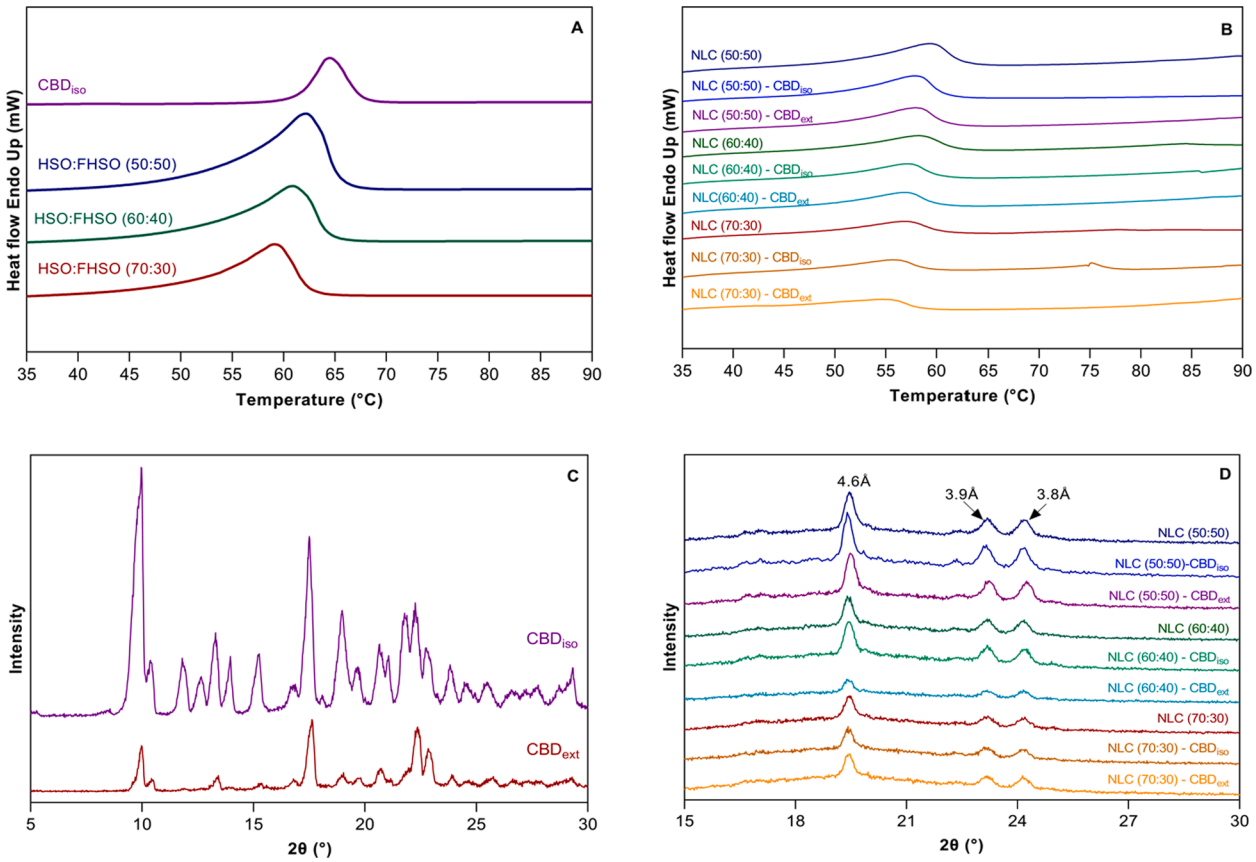

Fig. 3. DSC thermograms (A) and (B), XRD patterns (C) and (D) of individual components and loaded and unloaded NLC (HSO:FHSO). HSO: hemp seed oil; FHSO: fully hydrogenated soybean oil; CBD_{iso}: Isolate cannabidiol; CBD_{ext}: Broad-spectrum CBD distillate.

the value of PS remained in the same range to the unloaded NLC, reinforcing the idea of a greater interaction between CBD_{ext} and liquid lipid (HSO). Matarazzo et al. (2021) observed a reduction in the PS of CBD-loaded NLC (177 ± 3 nm) in comparison with the unloaded NLC (285 ± 5 nm) and related this result with the lipophilic characteristics of CBD that may have made the system more compact due to intermolecular interactions between the CBD and the nonpolar components of the NLC, which led to the retraction of the particles. However, it is interesting to observe that all CBD-loaded NLCs obtained in this study presented PS below 250 nm. Regarding the PDI, no significant differences were observed by the incorporation of CBD_{iso} and CBD_{ext} for all lipidic compositions evaluated (Table 1), remaining with values around 0.250.

3.2.1.2. Thermal melting behavior and polymorphism. The thermal properties of the NLCs were investigated to evaluate their crystallinity and the interactions between the lipids and CBD because they can impact not only the thermodynamic stability of the structures, but also the loading capacity and release of the bioactive compounds (Matarazzo et al., 2021; Zielińska et al., 2023). The CBD_{ext} and HSO presented no endothermic events under the conditions evaluated (results not shown). The DSC curves of the CBD_{iso} and the lipid mixtures on a macroscale are presented in Fig. 3a. The CBD_{iso} presented a single endothermic event at 64.5 °C, which corresponds to its melting point (T_m) (Table S3). The melting curves of the lipid mixtures also presented a single endothermic event corresponding to the T_m of the FHSO, which constitutes the solid lipid matrix of the mixture. As expected, the increase of HSO content in the lipid mixtures decreased their T_m and enthalpy change (ΔH) (Fig. 3a and Table S3), due to the predominant presence of triunsaturated TAGs with low melting point (around 87.8%) (Mungure & Birch, 2014), similarly to the behavior observed by Lüdtke et al. (2022) for the lipid systems obtained with different ratios between FHSO and HOSO.

The thermal behavior of the particles is influenced by the addition of other components to the system, such as the emulsifier and bioactive compound (Bunjes & Unruh, 2007). The unloaded-NLCs obtained with different HSO:FHSO proportions presented lower melting points than their respective lipid mixture (Fig. 3b and Table S4), probably a consequence of the addition of the emulsifier (SL) which can promote a crystallization of TAGs in a less compact structure than the lipid in macroscale. In addition, reductions in the ΔH values (Table S4) were also observed due to the lower concentration of lipids in the NLC suspensions (Lüdtke et al., 2022).

The values of T_m were in the range of 58.07 ± 0.09 °C – 59.32 ± 0.01 °C for NLC (50:50), 56.77 ± 0.11 °C – 58.26 ± 0.11 °C for NLC (60:40), and 55.11 ± 0.25 °C – 56.86 ± 0.16 °C for NLC (70:30). For all the lipid compositions evaluated, the T_m and ΔH of the NLCs decreased in the following order: unloaded-NLC > $NLC\text{-}CBD_{iso}$ > $NLC\text{-}CBD_{ext}$, which can be related to the presence of more amorphous regions in the CBD_{ext} (Fig. 3c) leading to a crystallinity reduction and consequent decrease in the T_m of the NLCs. Both CBD_{iso}- and CBD_{ext}-loaded NLCs presented single thermal events, similarly to the unloaded-NLCs, suggesting that after the incorporation into the NLCs, the bioactive compound was in an amorphous state or molecularly dispersed in the lipid matrix (Matarazzo et al., 2021).

The evaluation of the particles' polymorphism (crystal behavior) by XRD is a useful tool to determine the causes of the endothermal events observed in the thermal behavior analysis. In addition, the evaluation of the crystalline characteristics of lipid matrices provides important information about its potential to incorporate bioactive compounds, since the crystallinity of the structures directly impacts their entrapment efficiency (Lüdtke et al., 2022). Both CBD_{iso} and CBD_{ext} presented characteristic crystalline patterns in the region between 5° and 30° (Fig. 3c). As expected, CBD_{iso} showed peaks with higher intensity than CBD_{ext},

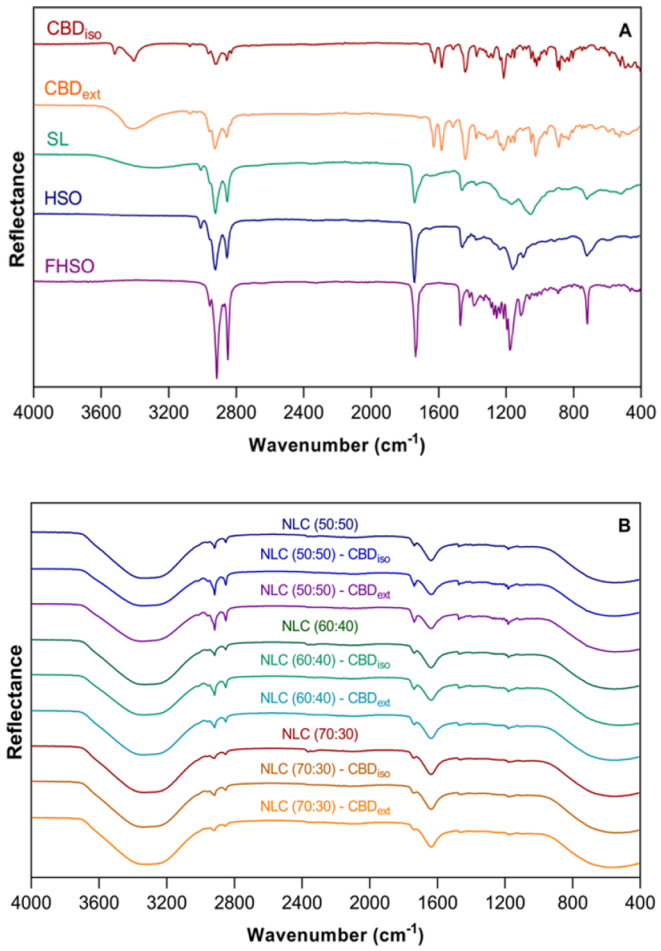

Fig. 4. FTIR spectra of individual (A) components and (B) NLC (HSO:FHSO). HSO: hemp seed oil; FHSO: fully hydrogenated soybean oil; CBD_{iso}: Isolate cannabidiol; CBD_{ext}: Broad-spectrum CBD distillate.

since the CBD_{iso} is available in the form of crystals with purity above 99.0%, while the CBD_{ext} has a honey-like aspect. Similar crystalline patterns of CBD_{iso} were reported in the literature (Alhadid et al., 2023; Hasan et al., 2023; Matarazzo et al., 2021). The crystalline peaks of both CBD_{iso} and CBD_{ext} were not detected in the CBD-loaded NLCs (Fig. 3d) indicating a good affinity between CBD and the lipid matrices, which corroborate the DSC results.

All NLCs presented reflection at 19.50°, 23.2° and 24.2° (2θ), corresponding to interplanar distances of 4.6 Å, 3.9 Å, and 3.8 Å, respectively, which characterizes the β polymorphic form (Azevedo et al., 2021). This is probably due to the homogeneity of fatty acids and TAG of the lipid matrix used to obtain the NLCs, since both HSO and FHSO are mainly composed by fatty acids with 18 carbon atoms (Alhadid et al., 2023; Lüdtke et al., 2022), which can facilitate the crystallization in the more stable β polymorphic form (Acevedo & Marangoni, 2010). The crystallization in the β form is desirable because at this stable polymorphic form, morphological modifications of the particles can be prevented, thus avoiding the expulsion of the bioactive compounds loaded into the structure (Lüdtke et al., 2022).

The intensity of the peaks of the NLCs decreased with the increase of HSO proportion in the lipid phase (Figure S1), due the presence of TAG of low melting point, which is also in agreement with the DSC results, where the T_m was found to decrease with the increase of HSO in the NLCs. Also, it can be noticed that the incorporation of CBD_{iso} into the NLCs slightly increased the intensity of peaks compared with the unloaded-NLCs, while for the CBD_{ext}-loaded-NLCs, it remained similar to the unloaded-NLCs, except for the NLC (60:40)-CBD_{ext} that presented the lowest peak intensity (Figure S1).

3.2.1.3. FTIR. FTIR spectra of the NLCs was obtained to identify the interactions that occurred between the constituents of the systems (Fig. 4). CBD_{iso} and CBD_{ext} presented very similar FTIR spectra, both with characteristic peaks at 1212 cm^{-1}, 1581 cm^{-1}, 1622 cm^{-2}, and 3401 cm^{-1}, compatible with their structure and in accordance with the literature (Matarazzo et al., 2021). The lipid matrices (HSO and FHSO), the emulsifier SL, the unloaded and loaded NLCs presented typical aliphatic peaks in the region of 2915 cm^{-1} and 2849 cm^{-1} for CH_2 asymmetrical and CH_2 symmetrical stretching vibrations, respectively, in addition to the peaks at 1736 cm^{-1} for C═O stretching vibration, which characterizes the lipidic fraction of the systems (Caldas et al., 2021; Shu et al., 2023; Siano et al., 2019). The peaks in the region around 3354 cm^{-1} present in all NLCs (O—H stretching) characterize the water content of the systems (Siano et al., 2019). The absence of new peaks and no significant changes on the characteristic peaks of the

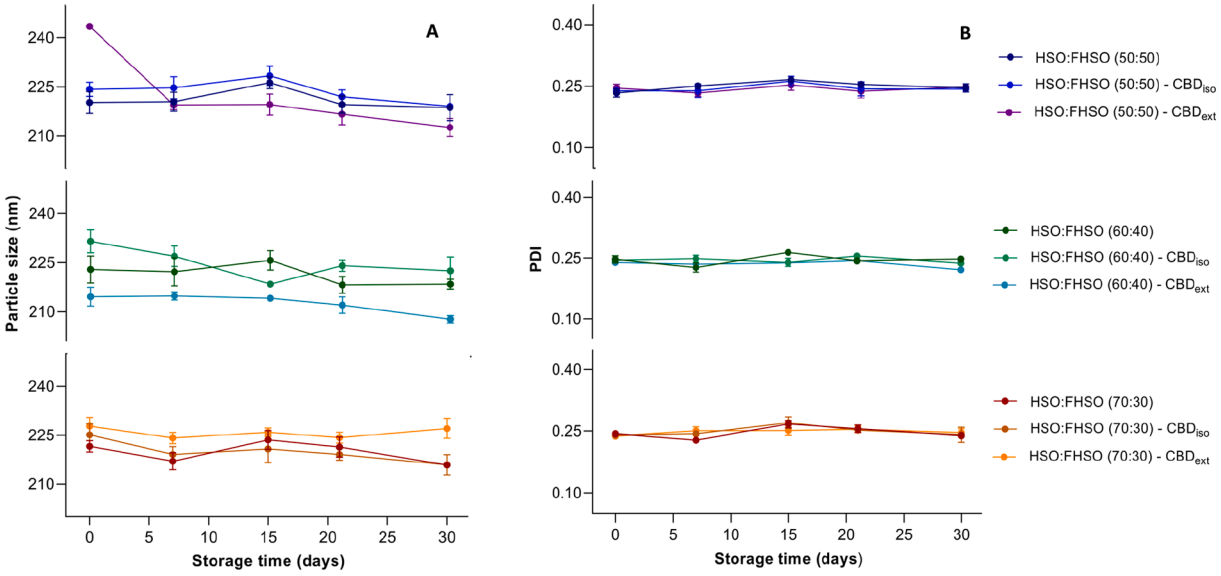

Fig. 5. Particle size expressed as z-average diameter (A) and polydispersity index (B) of the NLCs during 30 days of storage. HSO: hemp seed oil; FHSO: fully hydrogenated soybean oil; CBD_{iso}: Isolate cannabidiol; CBD_{ext}: Broad-spectrum CBD distillat.

loaded NLCs demonstrate that both CBD_{iso} and CBD_{ext} had good compatibility with the lipidic matrix and indicate the incorporation of the bioactive compounds into the structures. In addition, the absence of the characteristic CBD peaks in the spectra of the loaded NLCs, suggest that no free CBD was deposited on the surface of the NLCs (Matarazzo et al., 2021; Mendes et al., 2019).

3.2.1.4. Entrapment efficiency (EE). The EE of the NLCs refer to the amount of CBD entrapped into the structure. The CBD-loaded NLCs presented an EE of 99.99% for all the compositions evaluated. The presence of liquid lipids (mainly responsible for solubilizing the CBD) and the disorganized lipid matrix of the NLCs are factors that favor the high EE generally observed in NLCs (Matarazzo et al., 2021; Reddy et al., 2023). Other studies also reported high EE of CBD (>99%) into other lipid-delivery systems, such as NE (Banerjee et al., 2021) and SNEDDS (Cherniakov et al., 2017). In the present study, the NLCs were obtained using lipid systems containing HSO at ratios varying from 50 to 70%, which is an oil rich in C18:3 acid and presents a high affinity with CBD, resulting in a CBD solubility of 0.25 g CBD/g HSO (Alhadid et al., 2023). Alhadid et al. (2023) reported that high CBD solubility in C18:3 acid-rich oils, such as HSO, is probably due to the formation of intermolecular π-π interactions between the aromatic ring in the CBD structure and the three unsaturated double bonds in C18:3 acid. The CBD was incorporated into the NCLs at ratios between 0.20 g CBD/ g HSO for HSO:FHSO (50:50) and 0.14 g CBD/ g HSO for HSO:FHSO (70:30), i.e., below the maximum solubility of CBD in HSO, which may also contributed to the high EE of CBD into the NLCs found in this study. Zielińska et al., (2023) observed an EE of 70% for CBD-loaded NLC obtained with Compritol® 888 ATO as solid lipid and Miglyol® 812, composed of medium-chain triglycerides extracted from endosperms of palm oil and coconut plants, as liquid lipid, suggesting that the composition of the liquid lipid used to produce the NLCs can impact the EE of CBD.

3.2.2. Physicochemical stability of the NLCs

The NLCs were monitored during storage in terms of PS, PDI, retention rate of CBD, and thermal melting behavior. The PS and PDI results over the storage time are presented in Fig. 5 and Table 1. For the unloaded particles, the PS presented slight variations during the storage time, varying from 218.7 ± 3.9 nm to 226.3 ± 1.8 nm for NLC (50:50), from 218.2 ± 2.6 nm to 222.9 ± 4.2 nm for NLC (60:40), and from 215.8 ± 0.9 to 223.6 ± 3.0 nm for NLC (70:30). The NLC (50:50) and NLC (60:40) presented no significant differences in PS during the storage time, while NLC (70:30) presented a significantly lower value at 30 days of storage. For the loaded particles, when both CBD_{iso} and CBD_{ext} were added, the PS were significantly reduced during the storage time for all lipid compositions evaluated (Fig. 5a and Table 1). Regarding the PDI values, it is evident in Fig. 5b that the values remained practically constant during the storage time for all the systems evaluated, demonstrating the excellent stability of the NLCs over time, which could be attributed to a low system kinetic energy (Shu et al., 2023). These results are in agreement with those reported by Lüdtke et al., (2022), that demonstrated a good stability of NLCs produced with HOSO and FHSO as lipid matrices and SL as emulsifier during 60 days of storage.

The retention rate of CBD was 100% during the storage time for all systems evaluated. These results can be attributed to stable polymorphic form acquired by the NLCs after preparation (Fig. 3d), which was confirmed by the good thermal stability of the NLCs during the storage time (Table S4), thus maintaining the CBD entrapped into the structures (Shu et al., 2023).

4. Conclusions

Firstly, the evaluation of the most suitable emulsifier to produce NLCs based on HSO and FHSO as liquid and solid lipids, respectively, indicated that the use of the emulsifiers T80 and soybean SL individually was more effective to produce stable NLCs with smaller and narrower size distribution than the mixture T80:SL (50:50, w:w). It demonstrated that SL is a potential natural food grade emulsifier to obtain NLCs, corroborating the results reported by Lüdtke et al., (2022). All the evaluated lipid proportions of HSO and FHSO were suitable to produce CBD–loaded NLCs with PS below 250 nm, stable PDI around 0.250 and EE superior to 99% with a CBD retention rate of 100% during the storage time of 30 days, which can be explained by the crystallization of all nanostructures in the stable β polymorphic form, independently of the lipid composition used. The high EE observed in this study for the CBD-loaded NLCs obtained using HSO and FHSO as lipid matrices can represent an advance for the knowledge about the production of NLCs as delivery system of CBD, since NLCs obtained with medium-chain triglycerides (Miglyol ® 812) presented EE around 70% (Zielińska et al., 2023).

Although the increasing proportion of liquid lipid (HSO) in the NLC promoted a decrease in the T_m and ΔH, all NLCs presented melting temperature over the corporal temperature, demonstrating the potential of these NLCs as a vehicle to deliver CBD. In addition, it was observed that the different forms of the active compound (CBD_{iso} and CBD_{ext}) did not affect the physicochemical characteristics of the NLCs, which is an interesting result since it demonstrate that these lipid-based nanostructures can be used to deliver CBD independently of the form that it is available. Further studies to evaluate the performance of these nanostructures applied in food systems and their behavior during the gastrointestinal digestion will be needed to demonstrate the efficacy in a dietary context.

Contribution statement

Renata Vardanega: Conceptualization, methodology, data curation, writing – original draft, review & editing, funding acquisition. **Fernanda L. Lüdtke:** Conceptualization, methodology, data curation, writing –review & editing. **Luis L. Loureiro:** Conceptualization, methodology, data curation, writing –review & editing. **Raquel F. S. Gonçalves:** Conceptualization, writing – review & editing. **Ana C. Pinheiro:** Writing – review & editing. **António A. Vicente:** Conceptualization, funding acquisition, supervision, writing –review & editing, validation.

CRediT authorship contribution statement

Renata Vardanega: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft. **Fernanda L. Lüdtke:** Conceptualization, Data curation, Methodology, Writing – review & editing. **Luís Loureiro:** Conceptualization, Data curation, Methodology, Writing – review & editing. **Raquel F.S. Gonçalves:** Conceptualization, Writing – review & editing. **Ana C. Pinheiro:** Writing – review & editing. **António A. Vicente:** Conceptualization, Funding acquisition, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.138295.

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