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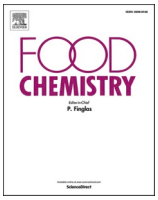

Stability of lutein-based nanoemulsions under thermal and UVC-light exposure: Effect of lutein esterification and oil phase constitution

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ABSTRACT

We evaluated the chemical and physical stabilities of lutein-delivery nanoemulsions and the effects of lutein esterification and oil phase composition. Oil-in-water nanoemulsions were formulated with free (LU) or esterified (LE) lutein, using soybean (S) or pumpkin seed (PS) oil, resulting in SLU, SLE, PSLU, and PSLE. Samples underwent physicochemical characterization and were stored under thermal (45 °C or 4 °C for 15 days) or UV-C light exposure (240 min). LU nanoemulsions showed greater chemical stability at 45 °C and under UV-C light than those with LE. LU nanoemulsions presented better stability at high temperature and under UV light compared to LE counterparts, particularly in S. At 4 °C, LE demonstrated improved stability in PS. PS-based nanoemulsions conferred enhanced protection, likely due to their antioxidant content. These findings challenge the common assumption that esterified carotenoids are inherently more stable and highlight the role of oil composition in carotenoid delivery systems.

1. Introduction

Lutein is a xanthophyll widely distributed in nature, recognized for its intense yellow-orange colour and potent antioxidant properties. Its consumption has been associated with beneficial effects on visual health, cognitive function, and the prevention of oxidative stress, which has promoted its incorporation into functional foods, supplements, and pharmaceutical products (Johnson, 2014; Miranda-Dominguez et al., 2022; Ranard et al., 2017; Zhang et al., 2024). However, its polyunsaturated structure and lipophilic character make it vulnerable to degradation by environmental factors such as light, oxygen, and heat, compromising its stability during processing and storage, and negatively affecting its bioavailability (Davidov-Pardo et al., 2016; Subagio et al., 1999).

To address these challenges, oil-in-water (O/W) nanoemulsion systems have been developed to encapsulate lipophilic compounds such as lutein, improve their dispersion in aqueous media, enhance protection against degradation, and potentially increase absorption (Qv et al., 2011; Teeranachaideekul et al., 2022; Weigel et al., 2018). Nevertheless, the effectiveness of these nanoemulsions depends on factors such as the type of oil and the chemical form of the encapsulated compound, making it essential to ensure their physical and chemical stability under different environmental conditions, such as light and temperature, for their application in the food and pharmaceutical industries (Qv et al., 2011; Teeranachaideekul et al., 2022; Weigel et al., 2018).

A critical factor influencing the stability and bioavailability of lutein is its chemical form. Various studies have shown that carotenoid esters, such as lutein diesters, exhibit greater resistance to thermal and photooxidative degradation compared to their free or monoesterified counterparts (Khachik & Beecher, 1988; Subagio et al., 1999; Yang et al., 2015). This enhanced stability may be attributed to the increased lipophilicity of esters, which facilitates their integration into cell membranes or the lipid matrix of the system, providing better protection against isomerization, oxidation, or cleavage (Mercadante et al., 2017; Subagio & Morita, 2003; Mínguez-Mosquera and Hornero-Méndez, 1994). Nonetheless, contradictory reports to the notion that esterification enhances the stability of carotenoids are also found. For instance,

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there are reports showing lower thermal stability of carotenoid esters, as observed in paprika oleoresins (***Capsicum annuum*** L.) (Pérez-Gálvez & Mínguez-Mosquera, **2002**) or pepper hybrids (Hetényi (***Capsicum frutescens***), Unikal (***C. annuum***) and & Unijol (***C. annuum*** X ***C. chinense***) subjected to various thermal drying protocols, where esterified xanthophylls degraded to a greater extent than their free forms (Se Souza et al., **2022**). These discrepancies may be due to the matrix, the type of carotenoid, the fatty acid profile involved in esterification, or specific processing conditions.

Additionally, although progress has been made in assessing the stability of lutein in nano-structured systems, most studies have focused on a single chemical form (usually the free form) under mild stress conditions. Wang et al. (**2023**) evaluated medium-chain triglyceride (MCT) oil nanoemulsions containing free lutein under thermal stress, although the maximum temperature tested was only 37 °C and no comparison was made with its esterified form. Similarly, Varma et al. (**2021**) studied liposomal systems loaded with free lutein under moderate thermal conditions, with a maximum temperature of 30 °C. Taking a broader approach, Zhang et al. (**2025**) investigated nanoparticles incorporating only the free form of lutein under thermal and photooxidative stress. On the other hand, although Gombač et al. (**2021**) compared emulsions containing free and esterified lutein, their study was limited to storage at 25 °C, without evaluating higher temperatures or UV radiation exposure. This highlights a significant gap in the literature: the lack of comparative studies assessing the simultaneous behavior of free and esterified lutein under combined thermal and photooxidative stress in nanoemulsion systems, limiting our understanding of their functional and commercial performance.

In general, most comparative studies have focused on a single stress condition, usually heat. Although esterification is generally believed to enhance the stability of carotenoids, some studies have shown that free lutein may exhibit greater resilience under certain conditions. Very few studies have systematically compared both forms of lutein under dual stress conditions (temperature and UV radiation) using standardized and controlled delivery systems. Moreover, the lipid phase of nanoemulsions, which significantly influences their stability against light and oxidation, remains an underexplored aspect. In particular, oils such as pumpkin seed oil (PSO), which is rich in tocopherols and phenolic antioxidants, may offer additional protection; however, its use in carotenoid-based nanoemulsions remains limited.

Therefore, this study addresses a critical gap in the literature by evaluating the combined effect of the chemical structure of lutein (free vs. dipalmitate ester) and the composition of the lipid phase (soybean oil vs. pumpkin seed oil) on the photostability and thermal stability of lutein-loaded nanoemulsions. The nanoemulsions were designed as simplified and chemically defined models, allowing the isolation of formulation effects and the accurate comparison of degradation profiles under controlled thermal (4 °C vs. 45 °C) and ultraviolet (UVC) light conditions. To the best of our knowledge, this is the first study to systematically evaluate the influence of carotenoid esterification and oil phase composition on the chemical and physical stability of lutein-based nanoemulsions under dual environmental stress, combining kinetic degradation modelling with multivariate analysis. These systems, composed of food- and pharmaceutical-grade ingredients, reflect formulation strategies applied in the industrial development of final products, ingredients, and excipients designed to disperse, stabilize, or enhance the bioavailability of carotenoids and other lipophilic compounds. Thus, they serve as representative models for investigating stability parameters with practical relevance. New insights into how to optimize lutein-based delivery systems to improve their stability will support their effective incorporation into functional foods, beverages, dietary supplements, and aqueous pharmaceutical formulations, considering their health-promoting properties.

2. Material and methods

2.1. Chemicals and material

(All-*E*)-lutein standard (95 % purity, HPLC), palmitic acid (hexadecanoic acid, 16:0), N,N′-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (99 %, DMAP) and Tween 20 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Two food-grade carrier oils were used as sources of long-chain triacylglycerides to prepare nanoemulsions. Soybean oil (Liza®, São Paulo, SP, Brazil) was purchased from a local supermarket (Brasília, DF, Brazil), whereas unrefined pumpkin seed oil was extracted from *Cucurbita maxima* seeds provided by Embrapa Hortaliças (Brasília, DF, Brazil) according to the procedure described by Lozada et al. (2021). The oxidative status and physicochemical characteristics of PSO are presented in this previous publication. Marigold flowers (*Tagetes* spp.) purchased from a local producer were cultivated and harvested in Brasília, DF, Brazil, in 2020. HPLC-grade methanol and methyl *tert*-butyl ether (MTBE) were purchased from J.T. Baker (Phillipsburg, NJ, USA) and other analytical-grade solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA). Samples and solvents were filtered, respectively, through Millipore membranes of 0.22 and 0.45 μm before HPLC analysis.

2.2. Extraction of lutein from marigold

Petals were manually removed from the marigold flowers, stored at −80 °C, and lyophilised (Beta 2–8 LDPlus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz-NI, Germany) for 24 h. Ground freeze-dried petals were subjected to the extraction with acetone that included partition (diethyl ether:petroleum ether, 1:1 (*v*/v)) and saponification (10 % (*w/v*) KOH in methanol, 16 h) steps, the latter also followed by washing (diethyl ether:petroleum ether, 2:1 (v/v)). The saponification step served as a purification stage by removing interfering compounds from the crude extract, such as triacylglycerols, chlorophyll derivatives, and other saponifiable lipids. This procedure to obtain the saponified extract containing primarily (all-*E*)-lutein in its free form was carried out as described by Rodrigues et al. (2019). The extract was concentrated in a rotary evaporator (Rotavapor® R II, Buchi, Valinhos-SP, Brazil; $T < 30$ °C), dried under N_2 stream, and stored at −80 °C until the chromatographic analysis, esterification reaction, or nanoemulsion preparation, described in Sections 2.5, 2.3 and 2.6, respectively.

2.3. Organic semi-synthesis of a lutein diester

The esterification reaction was conducted according to a methodology adapted from Tsao et al. (1995) and Young et al. (2007). Briefly, free lutein (added as the free lutein-rich extract obtained from marigold) solubilised in dry methylene chloride, and palmitic acid were mixed in the proportion of one to three moles in a round-bottom flask, under constant agitation at room temperature, protected from light and under an N_2 steam. In this reaction tube, 1,3-dicyclocarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) were added as a condenser and a catalyst, respectively. After 24 h, the mixture was dried on a rotary evaporator and redissolved in 48 mL of a mixture of acetonitrile/*tert*-butyl ether (1:1, *v*/v). The undissolved material was separated by centrifugation (4000 rpm, 5 min - Hettich Zentrifugen, Mikro 220R, Germany) and the supernatant containing esterified lutein collected, dried under N_2 and stored at −80 °C until spectrophotometric and chromatographic analyses.

2.4. Total lutein quantification by UV–visible (UV–vis) spectrophotometry

The total content of lutein in petal extracts, reaction products, and oil phases used to calculate emulsion formulation was estimated by spec-

trophotometry (Agilent 8453 Spectrophotometer, SC, USA). Whereas the lutein content is typically determined by using the specific extinction coefficient of lutein in ethanol ($E^{1\%}_{1cm} = 2550$) (Davies, 1976), this solvent does not dissolve adequately carotenoid esters and oils. In this case, petroleum ether was used and the extinction coefficient of lutein in this solvent was estimated from that of α-carotene, which possesses the same chromophore of lutein, according to Eq. 1 (Davies, 1976).

$$E^{1\%}_{1cm\ (A)} = E^{1\%}_{1cm\ (B)} \times \frac{mol.wt\ (B)}{mol.wt\ (A)} \quad (1)$$

Where $E^{1\%}_{1cm\ (A)}$ is the coefficient to be determined, $E^{1\%}_{1cm\ (B)}$ is the coefficient of α-carotene in petroleum ether (2800 - Davies, 1976), *mol.wt* (B) is the α-carotene molecular weight and *mol.wt* (A) is the lutein molecular weight.

Then, the scan UV–Vis absorption spectrum (300–600 nm) of each lutein-rich solution in petroleum ether was recorded using a diode array spectrophotometer and the total carotenoid content expressed as lutein (μg/mL) was estimated following the Lambert-Beer equation (Eq. 2).

$$[Total\ lutein]\mu g \Big/ mL = \frac{A \times 10^4}{E^{1\%}_{1cm}} \times V \times DF \quad (2)$$

where A, $E^{1\%}_{1cm}$, V and DF refer, respectively, to the absorbance measured at the maximum wavelength, absorption coefficient of lutein calculated for petroleum ether, solvent volume, and dilution factor.

2.5. Carotenoid analyses by HPLC-DAD-MS

The saponified extract of carotenoids from marigold petals and the product of the semi-synthesis reaction were analysed in a Shimadzu high-performance liquid chromatograph (HPLC) equipped with a quaternary pump (LC-20 CE), online degasser (DGU-20A5), autosampler (SIL-20 AC) and a diode array detector (DAD, SPD-M20A). The equipment was connected in series to a mass spectrometer containing a single quadrupole analyser and an atmospheric pressure chemical ionisation (APCI) source operating in positive mode (LCMS2020, Shimadzu - Kyoto, Japan). Carotenoids were separated on a C_{30} YMC column (5 mm, 250 × 4.6 mm i.d.) (Waters, MA, USA) kept at 35 °C, using the chromatographic conditions described for carotenoid ester analysis by Rodrigues et al. (2016). For comparison purposes, all the extracts were injected in the same conditions, at concentrations around 10 μg/mL. UV–Vis spectra were acquired between 280 and 700 nm, and chromatograms were processed at 445 nm. MS parameters were those described by Murador et al. (2019), with full MS spectra being acquired in the range of 200 to 1200 *m/z*.

Lutein and lutein esters were identified considering the chromatographic data (retention time and elution order on C_{30} phase), characteristics of the UV–Vis spectra (free and esterified forms of a xanthophyll share the same maximum absorption wavelength (λ max) and vibrational fine structure (% III/II)), and mass spectra features (protonated molecule $[M + H]^+$ and *in-source* fragments), as well as the co-chromatography with (all-*E*)-lutein standard and the comparison with literature data (Breithaupt et al., 2002; Rodrigues et al., 2016; Rodrigues et al., 2019). Quantification was performed using external analytical curves of commercial standard of (all-*E*)-lutein (1.5–46.2 μg/mL), and (all-*E*)-lutein dipalmitate (0.1–16.6 μg/mL) obtained via semi-synthesis.

2.6. Formulation of nanoemulsions

Four nanoemulsion formulations were prepared by combining each chemical form of lutein with one of two carrier oils. Free lutein, as the dried saponified extract of marigold, and lutein ester, as the dried esterification product, were each dissolved in HPLC-grade ethyl acetate using an ultrasonic bath to enhance dissolution. The resulting solutions were then separately incorporated into either soybean oil (SO) or pumpkin seed oil (PSO), yielding four distinct oil phases: free lutein in SO, lutein ester in SO, free lutein in PSO, and lutein ester in PSO. The organic solvent was removed under vacuum until the starting volume of oil was reached and no odour of solvent was perceived. The carotenoid concentration, estimated by UV–Vis (Section 2.3), was calculated to be 1 mg lutein/g oil, or 20 μg lutein/mL emulsion.

Regardless of the formulation, the aqueous phase was composed of ultrapure water containing 1 % (*w*/w) of surfactant Tween 20. A coarse oil-in-water (O/W) emulsion was prepared by mixing the oil phase (2 %, *w/v*) with the aqueous phase (98 %, *v*/v) using a high-speed mixer (Turrax M133/1281–0, Biospec Products Inc., Bartlesville-OK, USA) for 2 min at 8000 rpm. The resulting emulsion was immediately loaded into a high-pressure microfluidizer (Microfluidics 110S, Newton, MA, USA) for four cycles at 13.980 psi (Luo et al., 2017). According to the oil phase constitution and lutein form, four nanoemulsions were obtained: soybean oil with free lutein (SLU), soybean oil with lutein ester (SLE), pumpkin seed oil with free lutein (PSLU), and pumpkin seed oil with lutein ester (PSLE). Aliquots of each sample were taken for characterization analyses, and the remaining volume was subject to stability assays. The choice of oils was guided by both scientific relevance and research goals: soybean oil, widely used and well-documented for nanoemulsion studies, served as a reference carrier, while pumpkin seed oil was selected for its functional lipid profile and in alignment with ongoing research into the valorisation of *Cucurbita* spp. seed oils for nutraceutical applications (Lozada et al., 2021).

2.7. Nanoemulsion characterization

2.7.1. Physical aspects

Nanoemulsions were characterized in terms of their mean droplet size (hydrodynamic diameter), polydispersity index (PDI), and particle charge (ζ-potential). Droplet size and PDI were determined by dynamic light scattering, while ζ-potential was assessed via electrophoretic mobility, all using a Zetasizer (ZEN3690, Malvern Instruments, Malvern, UK). To minimize multiple scattering effects, samples were diluted (1:100, *v*/v) in purified water before measurement. Moreover, creaming (gravitational phase separation) eventually formed by physical instability was visually monitored (McClements, 2007).

2.7.2. Chemical aspects

The colour of the nanoemulsions was assessed using a HunterLab colourimeter (Hunter Colour Quest XE, VA, USA). Aliquots (6 mL) of each nanoemulsion were transferred to a quartz cuvette, and colour measurements were performed using illuminant D65, observer angle 10°, against a black background. The colour values L^* (lightness, white to black), a^* (red-green axis), and b^* (yellow-blue axis) were recorded in the CIELAB colour space. Subsequently, the hue angle (h), and chroma or colour saturation (C^*) were calculated from these chromaticity coordinates according to Eqs. 3 and 4, respectively:

$$h = tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (3)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (4)$$

Another relative value, colour difference (ΔE), was calculated from these coordinates to express perceptible colour changes between two time points during the stability assay (Section 2.8.1), as follows:

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \quad (5)$$

Additionally, the carotenoid composition of each nanoemulsion formulation, with emphasis on lutein forms and content, was assessed by HPLC-DAD-MS. For that, carotenoids were extracted following the method of Xavier et al. (2012), with modifications. Briefly, 8 mL of tetrahydrofuran were added to 2 mL of each emulsion, and the mixture

was vortexed for a few seconds. Carotenoids were then partitioned into diethyl ether:petroleum ether mixture (2:1, *v*/v), and the resulting ethereal extract was concentrated in a rotary evaporator, dried under N_2 flux and stored at −80 °C until chromatographic analysis, as described in Section 2.5.

2.8. Stability study

After production, each nanoemulsion formulation was aliquoted for chemical and physical characterization (Section 2.7), and these initial measurements were considered as time zero for the stability experiments. The remaining volume was then divided into four parts: two portions comprising approximately 1000 mL were immediately allocated for the thermal stability tests, the third portion was stored at refrigeration temperature (4 ± 1 °C) until the photostability assay, while the remaining portion was distributed in triplicate into multiple and previously labelled tubes for creaming evaluations.

2.8.1. Thermostability

One portion of each nanoemulsion stored in a glass flask wrapped in aluminium foil was kept at 45 ± 1 °C in a Biological Oxygen Demand (BOD) chamber (model EL202/4, Eletrolab, São Paulo, Brazil). The second portion was stored at 4 ± 1 °C, also protected from light, in a frost-free refrigerator (model RFCT 451, BSH Continental©, São Paulo, Brazil). Aliquots of the nanoemulsions were collected every three days over a 15-day period under both storage conditions to assess physical (mean droplet size, PDI, ζ-potential, and creaming) and chemical (carotenoid composition and colour) parameters, according to the methods described in Section 2.7. To monitor creaming, transparent test tubes containing each emulsion, stored in the dark under the two temperature conditions, were taken at each time point for visual observation and measurement of phase separation, if any (McClements, 2007).

2.8.2. Photostability

An aliquot of each nanoemulsion was transferred to transparent, labelled test tubes that were sealed and exposed in the horizontal position to ultraviolet (UV) radiation (30 W UV lamp, Philips, F30T8GL) inside a mirror chamber. The total experiment lasted 4 h, with the tubes corresponding to the triplicate of each sample were removed from the chamber at predetermined intervals (0, 30, 60, 120, and 240 min) for analyses of droplet size, PDI, ζ-potential and carotenoid composition. Control samples prepared by wrapping the test tubes in foil to protect them from light, were simultaneously exposed to the UV radiation, and removed for analyses at the same intervals as the exposed samples. The distance between the lamp and the test tubes (150 mm) was the same for all samples.

2.8.3. Kinetic modelling

The first-order kinetic model was adjusted to the data of the thermal degradation and photostability of the carotenoids (Eq. 6). Knowing the reaction rate constant (k), it was possible to calculate the half-life ($t_{\frac{1}{2}}$) of each nanoemulsion at different storage temperatures (Eq. 7).

$$ln_{(C_t)} = ln_{(C_0)} - kt \tag{6}$$

$$t_{\frac{1}{2}}t = \frac{ln2}{k} \tag{7}$$

where, C_0 and C_t refer to the carotenoid concentration (μg/mL) at zero time (initial) and t time, respectively, and k refers to the reaction rate constant.

2.9. Statistical analysis

One batch was prepared for each of the four nanoemulsion formulations. Each batch was then readily divided into three independent experimental units. For each of these units, the analyses were performed in triplicate, and the results are expressed as mean ± standard deviation. Differences among treatments were determined using a three-way analysis of variance (ANOVA) to determine the interaction between the type of nanoemulsion, time, and temperature/exposure to light. In addition, a one-factor ANOVA was performed within each nanoemulsion, followed by Tukey's post-hoc test with a 95 % confidence level. Statistical analyses were performed using the StatPlus v.5 software (AnalystSoft Inc., Canada). The regression and graphic construction analyses were performed with the GraphPad Prism 8.3.1 software, and chromatograms were built using Origin 8.5.

To evaluate the influence of temperature and UV light exposure on the physicochemical stability of lutein-rich nanoemulsions, Multiple Factor Analysis (MFA) was chosen due to its suitability for simultaneously analysing multiple data sets measured on the same observations, allowing investigation of interrelationships among experimental conditions and stability parameters. MFA integrates various groups of related variables into a unified statistical framework, facilitating a comprehensive understanding of the factors influencing nanoemulsion stability.

Two separate MFAs were performed to evaluate the stability under different conditions. The first MFA assessed the impact of UV light exposure, including active quantitative variables such as carotenoid content, hydrodynamic diameter (DH), polydispersity index (PDI), and zeta potential (ZP). Supplementary categorical variables included oil type (pumpkin seed oil, PSO; soybean oil, SO) and lutein chemical form (free or esterified). Time was considered a supplementary quantitative variable to aid interpretation without affecting the primary factor structure.

The second MFA evaluated the effect of storage temperature, encompassing active quantitative variables such as carotenoid content, DH, PDI, ZP, and colour parameters (lightness (L*), chroma (C*), hue angle (h), and colour difference (DE)). Supplementary categorical variables included storage temperature (4 °C or 45 °C), oil type (PSO or SO), and lutein chemical form (free or esterified). Similarly, storage time was treated as a supplementary quantitative variable.

Both MFAs involved constructing a concatenated matrix of the active variables, with each dataset weighted by the inverse of its first eigenvalue to ensure balanced contribution. Analyses were performed using XLSTAT 2017.2 software (Addinsoft, France), and results were interpreted through the distribution of factor scores and relative contributions of variables to each explanatory dimension.

3. Results and discussion

3.1. Organic semi-synthesis of a lutein ester

Lutein naturally occurs in marigold flowers predominantly in its esterified form. In the saponified extract obtained from marigold petals, (all-*E*)-lutein accounted for approximately 90 % of the total carotenoids (Fig. 2A). This lutein-rich extract was subjected to an acylation reaction with an excess of saturated long-chain fatty acid (palmitic acid, 16:0) by using an adapted procedure. The selection of palmitic acid was based on its natural abundance, analytical availability, and its relevance in native lutein esters from marigold petals, enabling the synthesis of diesters with physicochemical properties comparable to those naturally occurring (Rodrigues et al., 2016; Rodrigues et al., 2019). This straightforward process successfully yielded (all-*E*)-lutein dipalmitate in a purity level considered satisfactory for testing the hypothesis (75 %, Fig. 2B). No detectable formation of monoester isomers was observed. Additional steps of purification such as open column chromatography or crystallisation that could further improve the compound purity were not carried out in the present work. (See Fig. 1.)

In the mass spectrum, (all-*E*)-lutein exhibited a prominent *in-source* fragment ion $[M + H\text{-}18]^+$ at *m/z* 551, which was more intense than the protonated molecule $[M + H]^+$ detected at *m/z* 569. This fragmentation

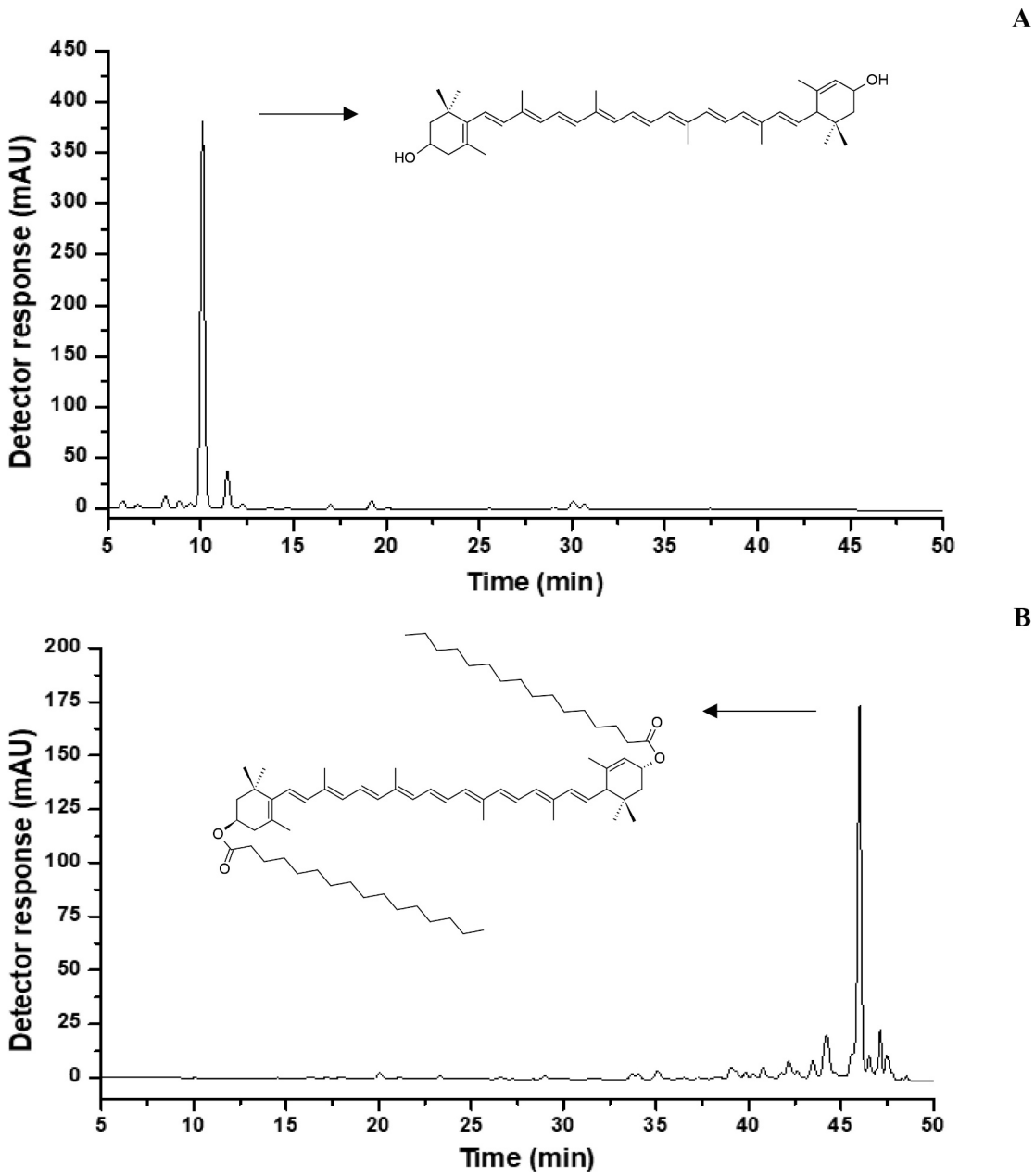

Fig. 2. Chromatograms, obtained by HPLC-DAD, of A. saponified extract of carotenoids from *Tagetes* spp., highlighting the peak of (all-*E*)-lutein at 10 min and B. the product of esterification reaction, highlighting the peak of (all-*E*)-lutein dipalmitate at 46 min. Chromatograms were processed at 445 nm.

pattern is characteristic of this xanthophyll since the loss of the hydroxyl group in allylic position to the double bond in the ε-ring is favoured. The peak eluting at approximately 10 min also showed maximum absorption wavelengths (λ_{max}) at 418, 445 and 471 nm, and a III/II of 58 %, consistent with the available standard and literature data (Britton et al., 2004; Rodrigues et al., 2016). As expected, the more nonpolar lutein diester eluted late on the reversed-phase C_{30} and displayed similar UV–Vis characteristics to those of free lutein. The detection of the *in-source* fragment ion $[M + H\text{-}256]^+$ at *m/z* 789 corroborated the peak assignment as (all-*E*)-lutein dipalmitate, corresponding to the facilitated loss of one palmitic acid moieties at 3′- position (Breithaupt et al., 2002; Rodrigues et al., 2016).

3.2. Characterization of nanoemulsions

The initial concentrations of lutein (either in free or esterified form, as appropriate for each formulation) determined by HPLC-DAD were 12.48 ± 0.55, 10.33 ± 0.44, 10.81 ± 0.05, and 9.54 ± 0.57 μg of lutein species per mL of nanoemulsion for PSLU, PSLE, SLU, and SLE, respectively (Fig. 3A and B). Since the initial lutein content can influence the stability, it is important to note that lutein content in nanoemulsions is within the same magnitude (Subagio et al., 1999). These actual concentrations were slightly lower than initially planned during pre-formulation, which might have resulted from physical losses during the nanoemulsion preparation process, and to the expected inherent differences between the UV–Vis spectrophotometry method used for lutein quantitation. UV–Vis spectrophotometry was used only for preliminary estimations of total lutein content in the extracts and oil phases, with the aim of guiding the development of the formulations. This method was not used for lutein quantification during nanoemulsion characterization, nor during the stability study. After the nanoemulsions were prepared, all quantitative analyses of lutein content during all over the experiment duration, from the beginning (zero time) to the end (final time) of the study, were carried out exclusively using HPLC-DAD, a more

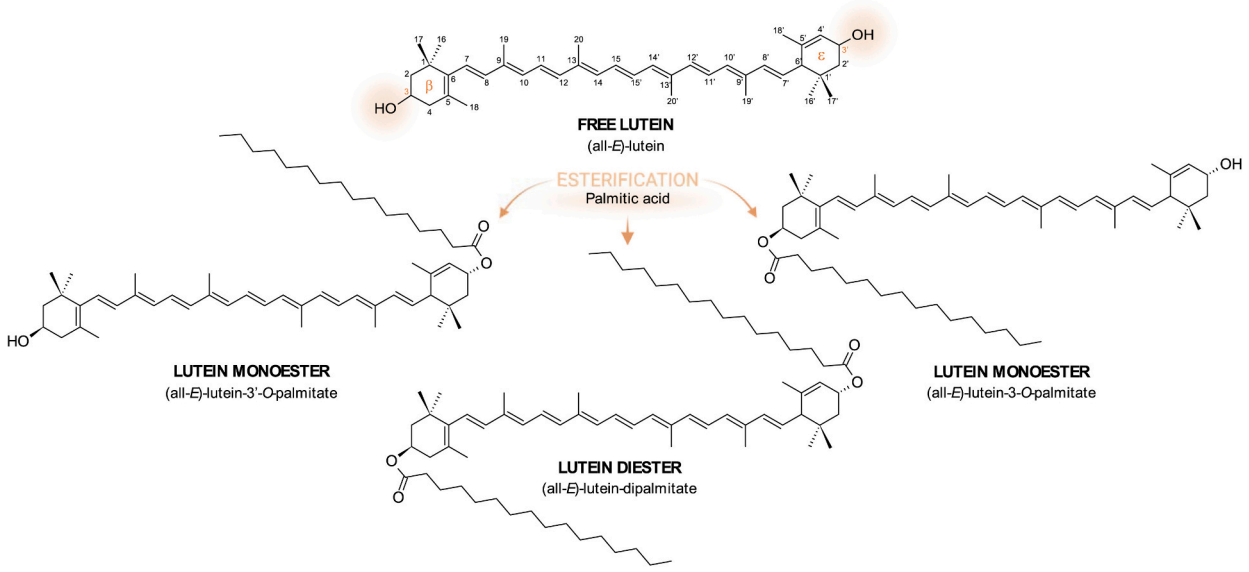

Fig. 1. Esterification products of (all-*E*)-lutein with palmitic acid. In lutein molecule, the hydroxyl groups at the 3- and 3′-positions on terminal β- and ε-rings, respectively, are highlighted.

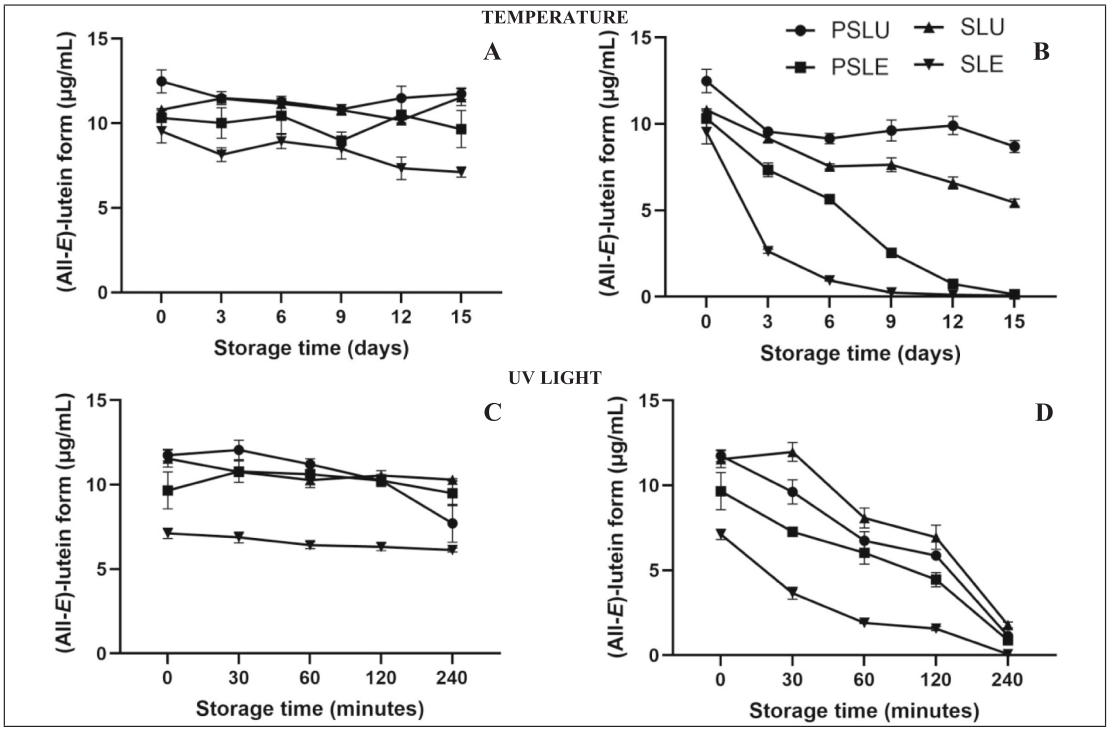

Fig. 3. Lutein content (µg/mL) in the four nanoemulsion formulations (PSLU ●, PSLE ■, SLU ▲, and SLE ▼) prepared with pumpkin seed (PS) or soybean (S) oils, containing either free lutein (LU) or lutein ester (LE), over the analysis period. To assess the effect of temperature, nanoemulsions were stored for 15 days at 4 °C (A) or 45 °C (B), with aliquots analysed every 3 days. To evaluate the impact of light, nanoemulsions were either protected (C, control) or exposed (D) to ultraviolet light for 240 min, with aliquots collected at four time points.

specific and accurate method. HPLC-DAD enabled the separation and individual quantification of compounds (free lutein and lutein dipalmitate), taking into account the composition of the mobile phase and using specific calibration curves for each analyte, as well as the purity of each standard in the calculations. Furthermore, for this study, it was essential to monitor the different lutein species separately throughout the stability assays. Minor chromatographic peaks corresponding to (*Z*)-lutein and (all-*E*)/(*Z*)-zeaxanthin isomers, in their free or esterified forms, were not included in this quantitation, so only the two markers (all-*E*)-lutein and (all-*E*)-lutein dipalmitate were monitored.

Initial colour assessment showed a* values close to neutral, at −0.34, −1.08, 0.03, and − 1.83 for PSLU, PSLE, SLU and SLE, respectively (Fig. 4A and F). The b* values, on the other hand, ranged between 44.81 and 45.52, indicating a clear yellow tendency (Fig. 4B and G). With positive L* values between 59.52 and 61.78, the calculated hue angles (h) ranged from 89.96° and 92.31° among all the formulations, characteristic of yellow hues (Fig. 4C, E, H and J). Colour saturation (C*) values were slightly below 50 in all samples (Fig. 4D and I). Thus,

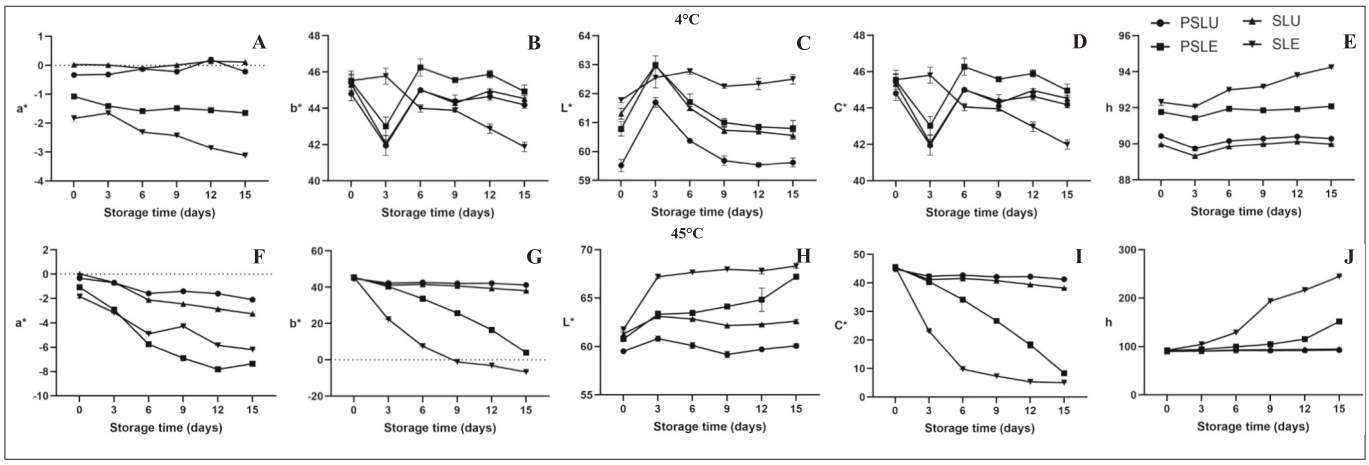

Fig. 4. Colour of nanoemulsion formulations (PSLU ●, PSLE ■, SLU ▲, and SLE ▼) prepared with pumpkin seed (PS) or soybean (S) oils, containing either free lutein (LU) or lutein ester (LE), over the analysis period. The colour coordinates and values in the CIELab space a* (redness), b* (yellowing), L* (lightness), C (chroma), and h (hue angle) were assessed each 3 days over a period 15 days of storage at 4 °C (A, B, C, D and E, respectively) or 45 °C (F, G, H, I and J, respectively).

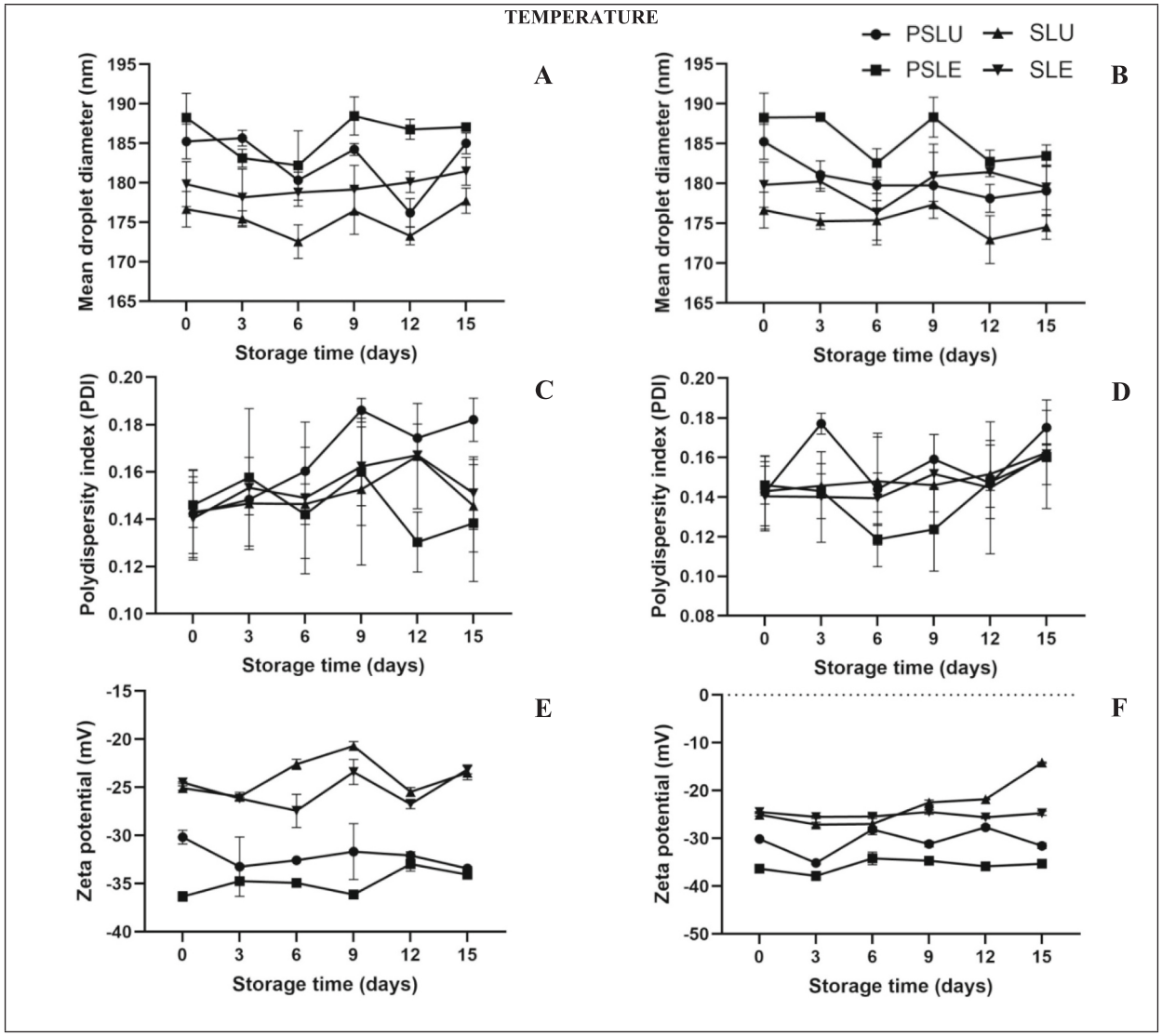

Fig. 5. Physical aspects of the four nanoemulsion formulations (PSLU ●, PSLE ■, SLU ▲, and SLE ▼) prepared with pumpkin seed (PS) or soybean (S) oils, containing either free lutein (LU) or lutein ester (LE), over the analysis period. Nanoemulsions were stored for 15 days at 4 °C (A, C and E) and 45 °C (B, D and F).

regardless of the oil type or lutein form, the freshly prepared nanoemulsions exhibited a consistent yellowish appearance with minimal differences among formulations. This is consistent with the presence of lutein in all samples at similar levels. Although unrefined PSO contains chlorophyll derivatives and other carotenoids contributing to its inherent colour (Lozada et al., 2021), its final concentration in the nanoemulsion was only 2 %, with findings indicating minimal influence on overall colour.

Initial physical characterization showed an average droplet size below 200 nm, ranging from 177 nm in SLU to 188 nm in PSLE, with a PDI of approximately 0.14, indicating monodisperse systems (Fig. 5A, B, C and D). The ζ-potential was −33.7, −30.2, −24.5, and − 25.1 mV for PSLE, PSLU, SLE, and SLU, respectively (Fig. 5E and F). The PSO-based nanoemulsions exhibited slightly higher negative surface charges compared to the SO-based formulations, which may contribute to enhanced electrokinetic stability, as suggested by Teo et al. (2015). ζ-potential values around or below −30 mV are generally associated with good colloidal stability due to strong electrostatic repulsion between droplets (Teo et al., 2015).

Since the photostability experiments were conducted at a later stage than the thermostability assay, a new characterization of the nanoemulsions was performed immediately prior to these experiments to ensure accurate baseline (time-zero) measurements before the UV exposure conditions. Therefore, the characterization presented above will be considered the baseline for the thermostability assay. For the photostability assay, the initial lutein concentrations were 11.75 ± 0.28, 9.66 ± 0.89, 11.54 ± 0.41, and 7.12 ± 0.25 μg/mL for PSLU, PSLE, SLU, and SLE, respectively (Fig. 3C and D), similar or slightly lower than those aforementioned.

Colour was not instrumentally measured in this case due to the small volume of the irradiated sample in each tube. The droplet size remained below 187 nm, with a maximum PDI of 0.18, indicating monodisperse systems (Fig. 6A and C). ζ-potential values were − 33.43, −34.07, −14.17, and − 23.13 mV for PSLU, PSLE, SLU, and SLE, respectively, showing a more pronounced difference in particle charge between PSO- and SO-based nanoemulsions compared to the initial characterization for thermostability (Fig. 6E). Values ≥ −30 mV are associated with improved long-term stability (Teo et al., 2015). No phase separation was visually noticed in any of the nanoemulsions at the start of both the experiments, consistent with their small droplet sizes and low PDI

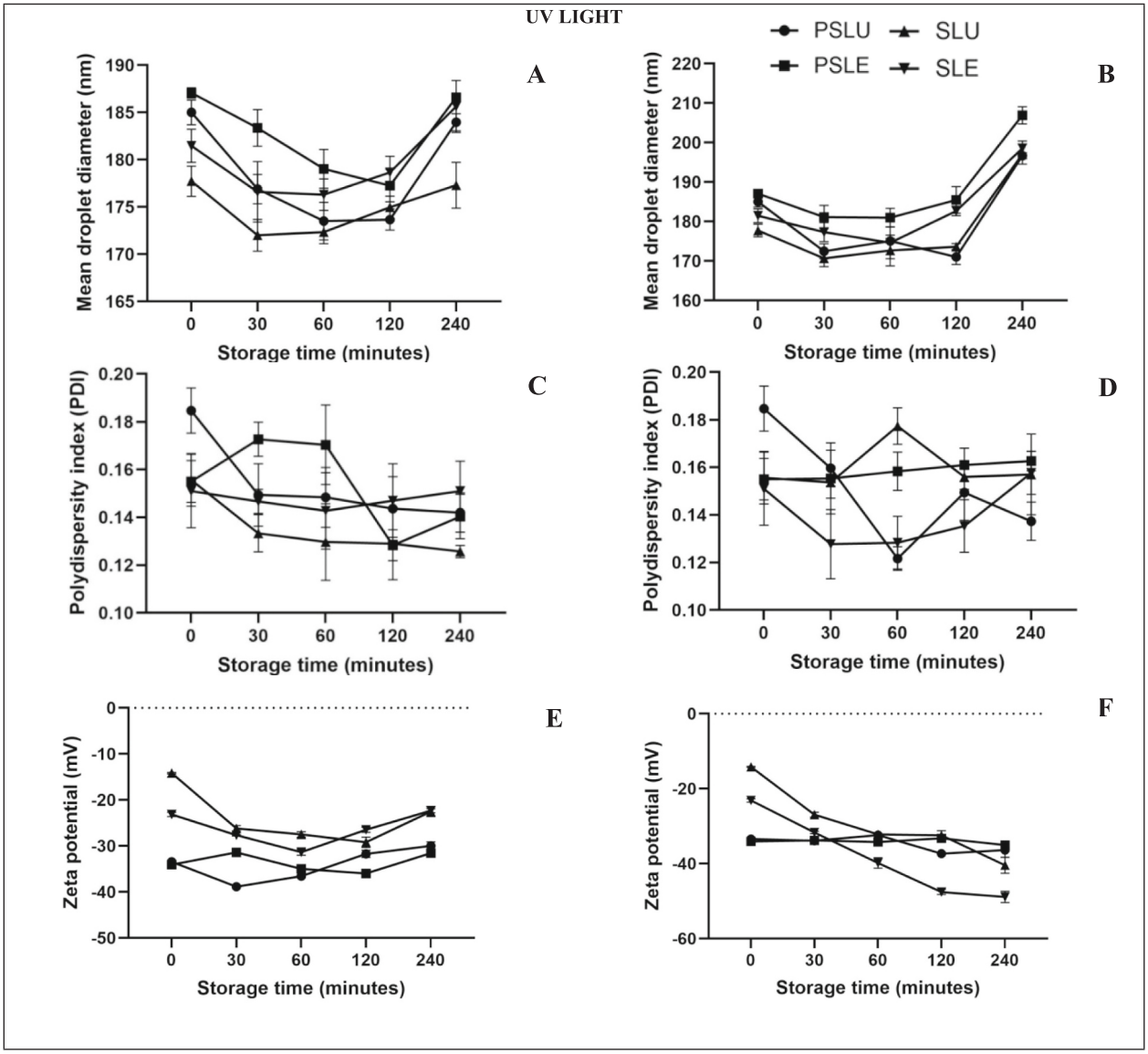

Fig. 6. Physical characterization of the four nanoemulsion formulations (PSLU ●, PSLE ■, SLU ▲, and SLE ▼) prepared with pumpkin seed (PS) or soybean (S) oils, containing either free lutein (LU) or lutein ester (LE), over the analysis period. Nanoemulsions protected (control, A, C and E) or exposed to ultraviolet light for 240 min (B, D and F).

values.

3.3. Impact of temperature on the chemical and physical stabilities of nanoemulsions

3.3.1. Degradation of free lutein and lutein ester from nanoemulsions

As shown in Fig. 3A and B, temperature significantly influenced the degradation of lutein in all nanoemulsions ($p < 0.05$). For SLU, lutein content remained relatively stable under refrigeration (4 °C) over the 15-day period. However, storage at 45 °C resulted in a sharp decline, with a final quantity of 5.44 μg/mL after 15 days, approximately 50 % of the initial amount. In the SLE formulation, lutein ester content remained stable until day nine at 4 °C, followed by a significant 23 % reduction by day 12 ($p < 0.05$). At 45 °C, degradation was markedly more pronounced, with a 72 % reduction within the first three days, culminating in a near complete loss (99 % reduction) by day 15, indicating the sensitivity of this formulation to mild-heat temperatures. Similarly, lutein ester was impacted by the temperature in the nanoemulsion prepared with pumpkin seed oil (PSLE) with a reduction of 98 % in its content at 45 °C by day 15 of storage, although stability was maintained under refrigeration. For PSLU, lutein content was also stable at 4 °C and showed only a 24 % decrease at 45 °C by day three, after which it remained relatively unchanged. Overall storage at 4 °C effectively delayed lutein degradation, both in its free and esterified forms, regardless of the oil phase composition. Chromatograms showing these lutein forms in nanoemulsions at zero and final times can be seen in Fig. S1. These results are consistent with previous studies, including those by Davidov-Pardo et al. (2016), and reinforce the established understanding that carotenoid degradation is accelerated by higher temperatures.

Kinetic modelling of lutein degradation in nanoemulsions under different storage temperatures fitted well to first-order reaction kinetics, with correlation coefficients (R^2) exceeding 0.70 (Table 1). As expected, the reaction rate constants were higher at 45 °C for all formulations, indicating a faster degradation rate of lutein at higher temperatures. Nonetheless, differences were noticed between formulations depending on the chemical form of lutein. Interestingly, nanoemulsions containing lutein dipalmitate (PSLE and SLE) exhibited higher reaction rate constants (0.2745 and 0.3351 $days^{-1}$, respectively), with lutein in SLE degrading more rapidly. In contrast, lutein was degraded at lower rates (0.0200 and 0.0420 $days^{-1}$ for PSLU and SLU, respectively) when incorporated in nanoemulsions in its free form, suggesting superior thermal stability of free lutein under the experimental conditions of this study. Even under refrigerated storage, the degradation rate of lutein ester in PSLE (0.0099 $days^{-1}$) was lower than in SLE (0.0173 $days^{-1}$), indicating a putative protective effect of PSO.

Table 1
Kinetic parameters and determination coefficients (R^2) for lutein degradation in lutein-delivery nanoemulsions at different storage temperatures or UV-light exposure.

Nanoemulsions	Temperature (°C)	Reaction rate constant k ($days^{-1}$)	$t_{1/2}$ (days)	R^2
PSLU	4	0.0099	70.01	0.7459
	45	0.0200	34.66	0.6978
PSLE	4	0.0041	169.06	0.9321
	45	0.2745	2.53	0.9031
SLU	4	0.0131	52.91	0.9724
	45	0.0420	16.50	0.9558
SLE	4	0.0173	40.07	0.7477
	45	0.3351	2.07	0.9780
Nanoemulsions	Exposure to UV light	Reaction rate constant k (min^{-1})	$t_{½}$ (min)	R^2
PSLU	Exposed	0.0095	72.96	0.9528
	Control	0.0019	364.81	0.9570
PSLE	Exposed	0.0097	71.46	0.9637
	Control	0.0006	1155.25	0.9715
SLU	Exposed	0.0080	86.64	0.9440
	Control	0.0004	1732.87	0.7284
SLE	Exposed	0.0184	37.67	0.9535
	Control	0.0006	1155.25	0.7772

PSLU: nanoemulsion prepared with pumpkin seed oil and free lutein; PSLE: nanoemulsion prepared with pumpkin seed oil and lutein ester; SLU: nanoemulsion prepared with soybean oil and free lutein; and SLE: nanoemulsion prepared with soybean oil and lutein ester. Nanoemulsions were stored over 15 days at 4 °C and 45 °C, and with lutein content being monitored each three days. Alternatively, nanoemulsions were irradiated with UV light or protected from irradiation (control samples) for 240 min, and with lutein content being monitored at specific intervals (0, 30, 60, 120 and 240 min).

Indeed, the type of oil used in the oil phase (PSO vs. SO) significantly influenced degradation rates. At 45 °C, SLE exhibited a reaction rate constant 1.22 times higher than PSLE, while SLU degraded twice as fast as PSLU. SO-based nanoemulsions generally showed faster degradation, with the lutein ester content decreasing threefold faster than in SLE compared to PSLE within the first three days. By day six, SLE had lost half of their lutein ester content, while PSLE retained a higher proportion. For free lutein, PSLU retained 70 % of their content by day 15 at 45 °C, while SLU retained only 50 %. At 4 °C, no significant differences were observed in the degradation of free lutein between emulsions with distinct oil types, but ester degradation reached 26 % in SLE, while it remained stable in PSLE.

These results may be attributed to the antioxidant-rich environment provided by PSO, which contains high levels of γ-tocopherol, β-sitosterol, and phenolic compounds (Boujemaa et al., 2020). In contrast, esterified lutein may be embedded deeper within the lipid phase, where it is more susceptible to degradation by lipid peroxidation products, especially under thermal stress. Free lutein showed lower degradation rates and longer half-lives, particularly in PSO-based systems. These findings suggest that lutein stability in nanoemulsions is critically influenced by lipid composition, molecular localization, and storage conditions (Teeranachaideekul et al., 2022; Zhang et al., 2025), challenging the generalized assumption that esterification inherently enhances carotenoid stability.

The potential role of chlorophyll derivatives present in PSO, which might act as photosensitizers or antioxidants depending on the conditions (Choe & Choe, 2013), could also be a contributing factor to the observed differences, although their overall concentration in the nanoemulsion was low. Higher temperatures are known to accelerate carotenoid degradation through oxidation, isomerization, and the formation of lower molecular weight compounds, consistent with findings by Zepka et al. (2009). Aman et al. (2005) reported increased levels of (*Z*)-lutein isomers with heat exposure, which negatively affected the antioxidant capacity. These changes occur as reversible thermal isomerization reactions proceed towards equilibrium.

The thermal stability of lutein in nanoemulsions is a critical parameter when designing delivery systems for food products that may be subjected to storage or transportation at these temperatures. Under the conditions of the present study, nanoemulsified lutein in its free form exhibited greater thermal stability than its esterified counterpart at 45 °C. This observation is contrary to the more widespread assumption and some empirical evidence suggesting greater stability of carotenoid esters, such as the findings by Ahmad et al. (2013) in bulk systems. While the stability of bioactive compounds is a complex phenomenon influenced by numerous variables, esterification is a commonly employed strategy to enhance stability, mostly by protecting reactive functional groups in their molecules. In a typical example, the phenolic hydroxyl group in vitamin E is esterified to form tocopheryl acetate, which exhibits reduced susceptibility to oxidation during processing and storage while maintaining bioavailability and biological activity in vivo (Desmarchelier et al., 2013). Similar approaches are used for labile vitamins such as ascorbic acid and retinol in both food and cosmetic applications (Guaratini et al., 2006). Nonetheless, as mentioned in the introduction, the literature presents some conflicting results regarding the stability of carotenoid esters in different systems (Arita et al., 2004; Mercadante et al., 2017), and potential analytical bias should also be

considered. Such bias may arise from the greater analytical complexity of carotenoid esters, and differences between free and esterified forms including solubility and stability in organic solvents, aggregation behavior, localization within heterogeneous systems, and molecular size, whereas they share the same UV–Vis spectra. In this regard, while we quantified the lutein species, it was not possible to directly determine the encapsulation efficiency of free lutein and lutein ester within the nanoemulsion droplets. Differently from analysing encapsulation in nanoparticles, in nanoemulsions carotenoids from both phases are dissolved, besides the difference in solubilization between free lutein and lutein diester in given solvents. Differences in encapsulation could potentially influence their exposure to degradation factors in the aqueous phase (Khalil et al., 2012). Moreover, the observed lower stability of lutein esters at higher temperatures might also stem from the ester bond to hydrolysis within the nanoemulsion environment, as suggested by Khalil et al. (2012) in other systems. Hydrolysis would release free lutein and palmitic acid, and the presence of free palmitic acid could potentially contribute to propagate oxidative reactions at elevated temperatures, accelerating the degradation of the remaining lutein species.

3.3.2. Effect of temperature on the colour of nanoemulsions

Lutein, characterized by its intense yellow colour, loses its colouration upon chemical degradation, a useful parameter for monitoring thermal oxidation and isomerization processes. The evolution of colour coordinates in PSO- and SO-based nanoemulsions stored at 4 °C and 45 °C is shown in Fig. 4. Nanoemulsions exhibited a* values near zero or negative (Fig. 4A and F). The negative a* values became more pronounced at 45 °C, towards a slight greenish hue, but remained relatively constant at 4 °C. PSO-based nanoemulsions tended to show more negative a* values than SO-based ones, likely due to the inherent colour of this oil. A similar relationship between oil type and a* values was observed by Steiner et al. (2018) in lutein nanoemulsions, with green-tinted grape seed oil contrasting with colourless medium-chain triglyceride oil.

The yellow colour intensity (b* value) remained relatively stable for most nanoemulsions at 4 °C, except for SLE, which decreased 1.1-fold by the end of the 15-day storage period (Fig. 4B and G). At 45 °C, lutein esters degraded significantly, with b* values for PSLE and SLE decreasing ~12- and ~ 7-fold, respectively. In contrast, free lutein nanoemulsions (PSLU and SLU) showed minimal changes, indicating better chemical stability under these conditions. Similar findings by Caballero and Davidov-Pardo (2020) reported a decline in b* values during storage at elevated temperatures, consistent with lutein oxidation.

Nanoemulsions of free lutein showed no significant changes in C* at either temperature, although a slight decrease was observed at 45 °C (Fig. 4D and I). In contrast, lutein ester nanoemulsions (PSLE and SLE) showed drastic reductions in C*, with PSLE and SLE decreasing 5- and 9-fold, respectively, at 45 °C. At 4 °C, SLE exhibited only a minor decrease (1.18-fold). These findings suggest that higher temperatures significantly reduce colour saturation in lutein ester nanoemulsions, indicating degradation and a shift towards less saturated colours, such as white, as carotenoids decompose (Ferreira & Spricigo, 2017). Temperature moderately affected lightness (Fig. 4E and J). PSLU and SLU nanoemulsions at 45 °C showed an increase in L* of up to 11 %, indicating lighter samples likely due to reduced yellow intensity and increased light reflection (McClements, 2002). Lutein ester nanoemulsions (PSLE and SLE) showed substantial increases in hue angles (h) by 1.65- and 2.71-fold (Fig. 4C and H), shifting towards less reddish and yellowish, respectively, which is indicative of lutein ester degradation. Overall, temperature significantly influenced colour fading, with changes in lightness, hue, saturation, and yellowing being more pronounced at 45 °C than at 4 °C. ΔE values were more pronounced at 45 °C as shown in Fig. S2. Lutein esters were particularly unstable under high temperatures, undergoing degradation through oxidation and isomerization of the conjugated polyene chain responsible for lutein's characteristic coloration (Boon et al., 2010; Davidov-Pardo et al., 2016).

Photographs of nanoemulsions during storage (Fig. S3) visually confirmed the fading of lutein esters, particularly in SLE, which became nearly colourless after 15 days at 45 °C. No phase separation was observed in any nanoemulsions during storage under either temperature. Free lutein emulsions showed greater visual colour stability compared to lutein esters at elevated temperatures. Nanoemulsions stored at 4 °C exhibited no noticeable colour changes, demonstrating that lower temperatures significantly mitigate carotenoid degradation, as corroborated by Khalil et al. (2012).

3.3.3. Effect of temperature on the physical stability of nanoemulsions

A significant interaction was observed between the type of oil (PSO or SO), lutein form (free or ester), storage time, and temperature for all parameters related to physical stability ($p < 0.05$). However, temperature did not significantly affect the hydrodynamic diameter of the particles, which remained below 200 nm throughout the 15-day storage period (Fig. 5A and B). Similarly, no significant changes in the polydispersity index (PDI) were observed by the end of storage on day 15 at either temperature (Fig. 5C and D). All nanoemulsions maintained PDI values below 0.2, indicating monodisperse systems regardless of storage conditions ($p < 0.05$).

The ζ-potential (Fig. 5E and F) at the end of storage varied across formulations and temperatures. At 4 °C, PSLU, PSLE, SLU, and SLE exhibited zeta potentials of approximately −33.43, −34.07, −23.50, and − 23.13 mV, respectively. At 45 °C, these values shifted to −31.56, −35.30, −14.16, and − 24.75 mV, respectively. Higher temperatures appeared to influence particle surface charges, particularly in SLU, where the zeta potential approached −14.16 mV at 45 °C, indicating a reduction in electrokinetic stability and potentially increased susceptibility to aggregation (Sari et al., 2015). This reduction in ζ-potential, particularly observed for SLU at 45 °C, might be associated with the lutein degradation and the generation of more polar degradation products that could affect the surface charge of the nanoemulsion droplets, or oxidation generating highly reactive radicals that destabilize the nanoemulsion system (Boon et al., 2010). However, the ζ-potential values for most formulations remained sufficiently negative to suggest reasonable physical stability throughout the study.

3.4. Impact of UV light on the chemical and physical stability of nanoemulsions enriched with lutein

3.4.1. Degradation of free lutein and lutein ester from nanoemulsions

The concentration of lutein in all nanoemulsions was significantly influenced by exposure to UV light, storage time, and the interaction between these factors ($p < 0.05$). Nanoemulsions exposed to UV light experienced substantial chemical degradation in a short period of time (Fig. 3C and D). PSLU and PSLE showed approximately 90 % degradation after 240 min of exposure, while SLU and SLE exhibited reductions of 84.59 % and 99.03 %, respectively. This degradation can be attributed to the generation of reactive oxygen species, such as singlet oxygen, by biological compounds upon UV irradiation. These species can then react with the highly unsaturated hydrocarbon chain of carotenoids, leading to their breakdown (Yahia & Ornelas-Paz, 2010). SLE showed a rapid 50 % reduction in carotenoid content within the first 30 min of UV exposure, indicating a high susceptibility of lutein ester in soybean oil to photodegradation. Conversely, SLU demonstrated relative stability (>95 % retention) during this initial interval but exhibited a gradual decline with continued exposure. Nanoemulsions protected from light (controls) retained up to 87 % of their initial lutein content over the 240-min period, clearly highlighting the detrimental effect of UV exposure on both free and esterified lutein. Chromatograms showing lutein and lutein ester peaks at the beginning and at the end of the photostability experiments are shown in Fig. S1, and the colour fading can be seen in Fig. S4.

The type of oil also played a crucial role in photostability. SO-based nanoemulsions (SLU and SLE) showed more pronounced degradation under UV exposure compared to the corresponding PSO-based nanoemulsions (PSLU and PSLE). PSO, being rich in bioactive compounds such as tocopherols and other antioxidants, likely provided a degree of protection against photooxidation, thereby minimizing lutein degradation. This aligns with findings by Mortensen and Skibsted (1999), who demonstrated that tocopherols inhibit the pro-oxidant effects of carotenoids under light exposure.

Table 1 presents the kinetic parameters for lutein degradation under UV exposure. Nanoemulsions exposed to UV light exhibited significantly higher reaction rate constants compared to their respective dark controls, indicating faster degradation. Notably, the highest degradation rate under UV exposure was observed in SLE (0.0184 min^{-1}), suggesting that lutein ester in SO is particularly susceptible to photodegradation. For PSO nanoemulsions exposed to UV light, the reaction rate constants for free and esterified lutein were similar (0.0095 and 0.0097 min^{-1}, respectively), indicating comparable behavior under these conditions.

Half-life data further revealed that non-exposed nanoemulsions were considerably more stable, with SLU showing a half-life of 1732.87 min compared to only 37.67 min for SLE under UV exposure. PSLU and PSLE exhibited similar half-lives under UV light (around 72 min), but these values increased substantially (to 364.81 and 1155.25 min, respectively) when the nanoemulsions were shielded from UV exposure. Similar trends have been reported for other carotenoids like lycopene, where light exposure significantly reduced the half-life (Ferreira & Rodriguez-Amaya, 2008).

Lutein's inherent susceptibility to degradation arises from its photosensitivity, with its conjugated double bonds being prone to isomerization, oxidation, and photobleaching upon exposure to light. UV radiation can trigger photooxidation reactions, producing radical carotenoid cations or enabling reactions between excited carotenoid states and other reactive species, ultimately leading to degradation (Boon et al., 2010; Konovalova et al., 2001). Nanoemulsions exposed to UV light showed noticeable fading of the characteristic yellow colour, particularly in the lutein ester formulations. As seen in Fig. 3B, SLE appeared nearly colourless after prolonged UV exposure, indicative of irreversible photobleaching due to extensive carotenoid oxidation. This phenomenon likely results from complex photochemical reactions involving the carotenoid molecule and potentially other components of the nanoemulsion system.

These findings emphasize the critical importance of understanding carotenoid degradation and oxidation mechanisms to enhance their stability in functional foods and other applications exposed to light. Strategies incorporating protective oils or antioxidants, such as the use of PSO rich in tocopherols, can effectively mitigate degradation and improve the delivery of lutein's functional and nutritional benefits in light-exposed matrices. Developing such strategies is crucial for maintaining the quality and efficacy of lutein-enriched products.

3.4.2. Impact of UV light on the physical stability of nanoemulsions

A significant interaction was observed between the type of nanoemulsion, storage time, and UV light exposure for all evaluated physical stability parameters ($p < 0.05$, likely from ANOVA). As shown in Fig. 6A and C, the average particle diameter did not significantly change in the final evaluation (240 min) for most treatments, remaining below 210 nm. However, UV light exposure tended to cause a slight increase in particle diameter across all nanoemulsions, with final values of 196.63, 206.90, 197.10, and 198.60 nm for PSLU, PSLE, SLU, and SLE, respectively, suggesting a potential for minor aggregation or droplet swelling upon UV irradiation.

The PDI remained below 0.2 throughout the UV exposure period for all formulations, confirming that the nanoemulsions maintained their monodisperse nature (Mudalige et al., 2019). Regarding ζ-potential (Fig. 6E), PSLU, SLU, and SLE exhibited a significant increase in the magnitude of their negative zeta potential by the end of the exposure period, reaching values of −36.40, −40.43, and − 48.87 mV, respectively. Slight variations were observed in the ζ-potential of PSLE nanoemulsions, but overall, UV light did not negatively impact the physical stability of the nanoemulsions as assessed by ζ-potential. The increase in the magnitude of the negative ζ-potential generally indicates enhanced electrostatic repulsion between droplets, which is associated with improved colloidal stability (Mudalige et al., 2019). This increase might be due to the formation of charged degradation products upon UV irradiation that adsorb onto the droplet surface, increasing the surface charge density.

3.5. MFA

The Multiple Factor Analysis (MFAs) were conducted to simultaneously assess the impact of temperature and UV light exposure on the physicochemical stability of lutein-rich nanoemulsions, providing insights into the role of oil types and lutein forms on the findings. For both MFAs, Factor 1 (F1) and Factor 2 (F2) explained substantial data variability, with F1 and F2 explaining 41.74 % and 25 % for temperature, and 34.38 % and 23.09 % for UV light, respectively (Fig. 7).

In the temperature-focused MFA (Fig. 7A), carotenoid content showed the strongest negative correlation to F1, evidenced by the degradation effect of higher temperatures (45 °C). Colour parameters were highly responsive to temperature changes, as indicated by the significant contributions of colour difference variables (ΔE, L, C, and H) to F1. Oil type and lutein form were strongly associated with F2, underscoring the differences in stability between nanoemulsions formulated with soybean oil (SO) and pumpkin seed oil (PSO), as well as between free and esterified lutein. PSO-based nanoemulsions exhibited greater stability, aligning positively with F2.

Regarding photostability (Fig. 7B), the MFA indicated that Factor 1 and Factor 2 accounted for 34.38 % and 23.09 % of the total variability, respectively. Oil type (PSO vs. SO) was notably influential, showing clear differentiation along F2. Pumpkin seed oil nanoemulsions showed better stability under UV conditions, likely due to antioxidant compounds like tocopherols present in PSO, thus effectively reducing oxidative damage. Similarly, lutein form (ester versus free lutein) strongly contributed to F1, where esterified lutein showed higher susceptibility to UV-induced degradation. This degradation was accompanied by a considerable decline in carotenoid content, while free lutein exhibited more resilience under UV exposure.

Overall, the MFAs revealed that both temperature and UV light substantially influenced the physical and chemical stability of nanoemulsions. Elevated temperatures and UV exposure accelerated carotenoid degradation, reflected by significant changes in droplet size, zeta potential, and particularly pronounced colour losses in lutein ester formulations. These findings emphasize the protective role of pumpkin seed oil and the comparatively higher stability of free lutein in nanoemulsion systems under stressful conditions. Collectively, these results provide valuable guidance for formulating nanoemulsions to enhance lutein stability, suggesting optimised industrial applications in functional food products and pharmaceuticals.

While this study provides fundamental insights into the stability of lutein in model nanoemulsion systems, future research is warranted to explore their application in more complex, real food matrices, where numerous intrinsic factors may interact with the delivery system. Further investigations into the long-term stability of lutein-loaded nanoemulsions or nanoparticles under a broader range of storage conditions, as well as mechanistic and applied bioavailability studies of encapsulated lutein forms, will be critical to support practical application. Additionally, exploring the influence of different fatty acid moieties in lutein esters on nanoemulsion stability may offer valuable strategies for formulation optimization. Beyond their physicochemical characterization, the nanoemulsions developed in this study exhibit practical potential for incorporation into a wide range of functional food products. Their stability at refrigeration temperature (4 °C) and ability

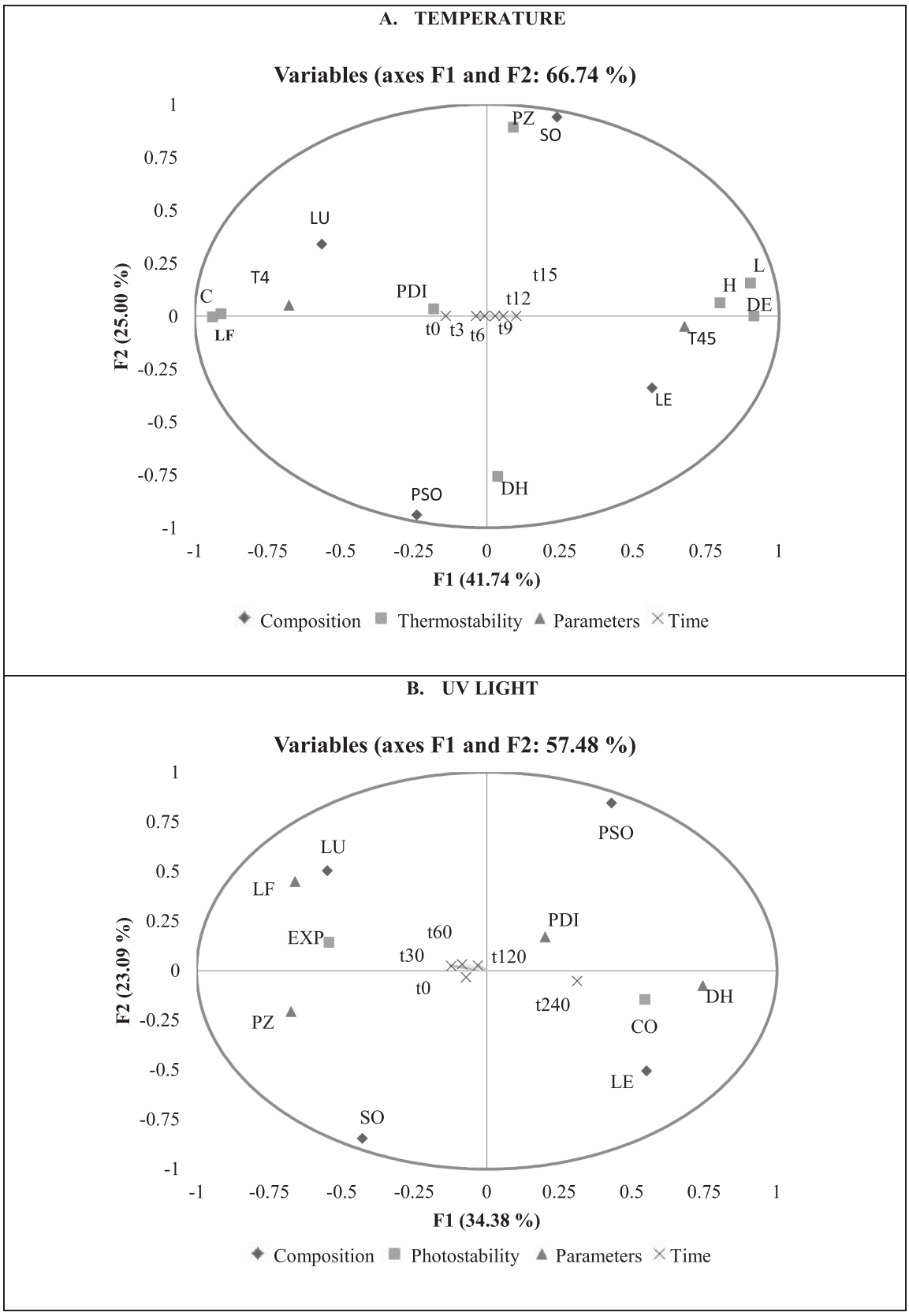

Fig. 7. The Multiple Factor Analysis of impact of temperature and exposed to ultraviolet light on the chemical and physical stability of nanoemulsions. Variables: Composition(The nanoemulsion composition): pumpkin seed oil (PSO), soy oil (SO), lutein free (LU) and lutein ester (LE); Photo-stability: Exposed a UV light (EXP) and Control (CO); Thermostability: storage at 45 °C (T45) and storage at 4 °C (T4); Parameters (physicochemical parameters): The concentration of lutein form (LF), Polydispersity index (PDI), The zeta potential (PZ), Hydrodynamic diameter of the particles (DH); Time (Storage time): For temperature: 0 day (t0), 3 days (t3), 6 days (t6), 9 days (t9), 12 days (t12) and 15 days (t15). For UV light: 0 min (t0), 30 min (t30), 60 min (t60), 120 min (t120), 240 min (t240).

to disperse lutein in aqueous systems render them suitable for applications such as plant-based beverages, smoothies, fortified juices, low-fat sauces, refrigerated spreads, yogurts, and dairy alternatives. The use of pumpkin seed oil as the oil phase not only enhances chemical stability through its antioxidant-rich composition but also contributes nutritional value, reinforcing the appeal of these systems for health-oriented formulations. Its superior performance can be attributed to the presence of natural antioxidants, such as tocopherols, polyphenols, and phytosterols, which create a favorable oxidative environment for lutein stability, as previously reported by Lozada et al. (2021). The selection of a low-chlorophyll variety (*C. maxima*) further minimized photooxidative effects, ensuring improved protection of encapsulated compounds. Furthermore, the distinct degradation behavior observed between free and esterified lutein under thermal and UV stress suggests tailored use cases: nanoemulsions with free lutein may be better suited for formulations exposed to light, whereas esterified lutein may be preferable in contexts requiring controlled release or limited light exposure. Importantly, these systems also deliver a stable yellow-orange coloration, which can enhance the sensory profile of products such as desserts and plant-based egg analogues, providing both aesthetic and functional benefits.

4. Conclusion

This study provides valuable insights into the chemical and physical stability of lutein-delivery nanoemulsions, highlighting the significant influence of both lutein esterification and the oil phase constitution under thermal and UV stress. Contrary to the general expectation of enhanced stability for carotenoid esters, our findings demonstrated that under temperature of 45 °C or UV-light exposure, and in the conditions employed in this study, free lutein exhibited superior thermal stability compared to lutein dipalmitate in both SO- and PSO-based nanoemulsions. Degradation data fitted well with a first-order kinetic model, with SLE showing the shortest shelf life, 2 days at 45 °C and 37.67 min under UV light, indicating its susceptibility to adverse conditions. This counterintuitive finding suggests that the esterification might, under specific conditions within the nanoemulsion environment, render lutein more susceptible to degradation, potentially through mechanisms such as hydrolysis or the pro-oxidant effects, or even due to a suboptimal oil-to-ester ratio for effective encapsulation and protection. Conversely, at refrigeration temperatures (4 °C), lutein ester showed improved stability in PSO, underscoring the complex interplay of temperature and formulation on carotenoid preservation.

Furthermore, the choice of oil phase significantly impacted lutein stability. Nanoemulsions formulated with PSO generally exhibited greater chemical stability for both free and esterified lutein under both thermal and UV stress compared to those prepared with SO. This protective effect of PSO is likely associated with its inherent antioxidant properties, not only due to the considerable presence of tocopherols (Lozada et al., 2021), but also to a synergistic interplay with other minor constituents such as phenolic compounds, phytosterols, squalene, and endogenous carotenoids. Together, these compounds may have contributed to an antioxidant microenvironment, which effectively minimized carotenoid degradation, particularly under UV irradiation. In nanoemulsions, the interface is often enriched with antioxidants from the oil phase, such as tocopherols and phenolics in pumpkin seed oil, which may have acted synergistically with free lutein located in this region to enhance protection. In contrast, lutein dipalmitate, being highly lipophilic, was likely confined to the oil core, where it may be more exposed to lipid peroxides, particularly in unsaturated oils like soybean oil. As a result, under forced stress, in the specific conditions used in the present study, free lutein could have outperformed its esterified form in terms of stability. The observed differences in stability have significant implications for the design and application of lutein-enriched products in the food and pharmaceutical industries. Formulators must carefully consider the intended storage conditions and the specific chemical form of lutein to maximize its preservation and efficacy. While this study provides fundamental insights into the stability of lutein in model nanoemulsion systems, future research is warranted to explore their application in more complex, real food matrices, where numerous intrinsic factors may interact with the delivery system. Notably, this work pioneers the use of pumpkin seed oil (PSO) as a promising oil phase in the formulation of carotenoid-based nanoemulsions. Its rich composition in antioxidant compounds, such as tocopherols, phytosterols, and phenolics, was shown to effectively stabilize both free and esterified lutein, particularly under UV stress, highlighting its potential as a functional ingredient in lipid-based delivery systems. Moreover, by revealing that free lutein, a more bioavailable form, can exhibit superior stability under thermal and photonic stress when appropriately encapsulated, this study challenges the conventional preference for esterified carotenoids and opens new perspectives for the development of stable, high-performance delivery systems that enhance both the efficacy and shelf life of bioactive compounds.

CRediT authorship contribution statement

Maria Isabel Ordoñez Lozada: Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation. **Daniele Bobrowski Rodrigues:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Iriani Rodrigues Maldonade:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Talita de Almeida Fernandes:** Validation, Methodology, Formal analysis, Data curation. **Ernandes Rodrigues de Alencar:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation. **Lívia de Lacerda de Oliveira:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Methodology, Data curation.

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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.

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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.2025.145401.

Data availability

Data will be made available on request.

References

Ahmad, F. T., Asenstorfer, R. E., Soriano, I. R., & Mares, D. J. (2013). Effect of temperature on lutein esterification and lutein stability in wheat grain. *Journal of Cereal Science, 58*(3), 408–413. https://doi.org/10.1016/j.jcs.2013.08.004

Aman, R., Schieber, A., & Carle, R. (2005). Effects of heating and illumination on *trans–cis* isomerization and degradation of β-carotene and lutein in isolated spinach chloroplasts. *Journal of Agricultural and Food Chemistry, 53*(24), 9512–9518. https://doi.org/10.1021/jf050926w

Arita, S., Otsuki, K., Osaki, K. I., Murata, Y., Shimoishi, Y., & Tada, M. (2004). Reduction in photostability by the esterification of β-cryptoxanthin. *Bioscience, Biotechnology, and Biochemistry, 68*(2), 451–453. https://doi.org/10.1271/bbb.68.451

Boon, C. S., McClements, D. J., Weiss, J., & Decker, E. A. (2010). Factors influencing the chemical stability of carotenoids in foods. *Critical Reviews in Food Science and Nutrition, 50*(6), 515–532. https://doi.org/10.1080/10408390802565889

Boujemaa, I., El Bernoussi, S., Harhar, H., & Tabyaoui, M. (2020). The influence of the species on the quality, chemical composition and antioxidant activity of pumpkin seed oil. *OCL, 27*, 40. https://doi.org/10.1051/ocl/2020031

Breithaupt, D. E., Wirt, U., & Bamedi, A. (2002). Differentiation between lutein monoester regioisomers and detection of lutein diesters from marigold flowers (*Tagetes erecta* L.) and several fruits by liquid chromatography–mass spectrometry. *Journal of Agricultural and Food Chemistry, 50*(1), 66–70. https://doi.org/10.1021/jf010970l

Britton, G., Liaaen-Jensen, S., & Pfander, H. (Eds.). (2004). *Carotenoids: handbook* (p. 647p). Birkhäuser Verlag: Basel.

Caballero, S., & Davidov-Pardo, G. (2020). Comparison of legume and dairy proteins for the impact of Maillard conjugation on nanoemulsion formation, stability, and lutein color retention. *Food Chemistry, 38*, Article 128083. https://doi.org/10.1016/j.foodchem.2020.128083

Choe, J., & Choe, E. (2013). Effects of chlorophyll addition and light on the oxidative stability and antioxidant changes of perilla oil emulsion. *Korean Journal of Food and Cookery Science, 29*, 53–68. https://doi.org/10.9724/kfcs.2013.29.1.53

Davidov-Pardo, G., Gumus, C. E., & McClements, D. J. (2016). Lutein-enriched emulsion-based delivery systems: Influence of pH and temperature on physical and chemical stability. *Food Chemistry, 196*, 821–827. https://doi.org/10.1016/j.foodchem.2015.10.018

Davies, B. H. (1976). Carotenoids. In T. W. Goodwin (Ed.), *Chemistry and biochemistry of plant pigments* (pp. 38–165). London: Academic Press.

Desmarchelier, C., Touvier, M., Lioret, S., Aubin, F., Galan, P., Hercberg, S., & Latino-Martel, P. (2013). The distribution and relative hydrolysis of tocopheryl acetate in the different matrices coexisting in the lumen of the small intestine during digestion could explain its low bioavailability. *Molecular Nutrition & Food Research, 57*(7), 1235–1243. https://doi.org/10.1002/mnfr.201200793

Ferreira, J. E. M., & Rodriguez-Amaya, D. B. (2008). Degradation of lycopene and β-carotene in model systems and in lyophilized guava during ambient storage: Kinetics, structure, and matrix effects. *Journal of Food Science, 73*(8), C589–C594. https://doi.org/10.1111/j.1750-3841.2008.00919.x

Ferreira, M. D., & Spricigo, P. C. (2017). Colorimetria - princípios e aplicações na agricultura. In M. D. Ferreira (Ed.), *Instrumentação pós-colheita em frutas e hortaliças* (pp. 209–220). Embrapa Instrumentação.

Gombač, Z., OsojnikCrnivec, I. G., Skrt, M., Istenič, K., Knez Knafelj, A., Pravst, I., & Poklar Ulrich, N. (2021). Stabilisation of lutein and lutein esters with polyoxyethylene sorbitan monooleate, medium chain triglyceride oil and lecithin. *Foods, 10*, 500. https://doi.org/10.3390/foods10030500

Guaratini, T., Gianeti, M. D., & Campos, P. M. (2006). Stability of cosmetic formulations containing esters of vitamins E and a: Chemical and physical aspects. *International Journal of Pharmaceutics, 327*(1–2), 12–16. https://doi.org/10.1016/j.ijpharm.2006.07.015

Johnson, E. J. (2014). Role of lutein and zeaxanthin in visual and cognitive function throughout the lifespan. *Nutrition Reviews, 72*(9), 605–612. https://doi.org/10.1111/nure.12133

Khachik, F., & Beecher, G. R. (1988). Separation and identification of carotenoids and carotenol fatty acid esters in some squash products by liquid chromatography. 1. Quantification of carotenoid and related esters by HPLC. *Journal of Agricultural and Food Chemistry, 36*(5), 929–937. https://doi.org/10.1021/jf00082a021

Khalil, M., Raila, J., Ali, M., Islam, K. M. S., Schenk, R., Krause, J. P., & Rawel, H. (2012). Stability and bioavailability of lutein ester supplements from *Tagetes* flower prepared under food processing conditions. *Journal of Functional Foods, 4*(3), 602–610. https://doi.org/10.1016/j.jff.2012.03.006

Konovalova, T. A., Gao, Y. L., Schad, R., Kispert, L. D., Saylor, C. A., & Brunei, L. C. (2001). Photooxidation of carotenoids in mesoporous MCM-41, Ni-MCM-41 and Al-MCM-41 molecular sieves. *The Journal of Physical Chemistry B, 105*(31), 7459–7464. https://doi.org/10.1021/jp0108519

Lozada, M. I. O., Maldonade, I. R., Rodrigues, D. B., Santos, D. S., Sanchez, B. A. O., de Souza, P. E. N., … de Lacerda, L. P. O. (2021). Physicochemical characterization and nano-emulsification of three species of pumpkin seed oils with focus on their physical stability. *Food Chemistry, 343*, Article 128512. https://doi.org/10.1016/j.foodchem.2020.128512

Luo, X., Zhou, Y., Bai, L., Liu, F., Deng, Y., & McClements, D. J. (2017). Fabrication of β-carotene nanoemulsion-based delivery systems using dual-channel microfluidization: Physical and chemical stability. *Journal of Colloid and Interface Science, 490*, 328–335. https://doi.org/10.1016/j.jcis.2016.11.057

McClements, D. J. (2002). Theoretical prediction of emulsion colour. *Advances in Colloid and Interface Science, 97*(1–3), 63–89. https://doi.org/10.1016/s0001-8686(01)00047-1

McClements, D. J. (2007). Critical review of techniques and methodologies for characterization of emulsion stability. *Critical Reviews in Food Science and Nutrition, 47*, 611–649. https://doi.org/10.1080/10408390701289292

Mercadante, A. Z., Rodrigues, D. B., Petry, F. C., & Mariutti, L. R. B. (2017). Carotenoid esters in foods: A review and practical directions on analysis and occurrence. *Food Research International, 99*, 830–850. https://doi.org/10.1016/j.foodres.2016.12.018

Mínguez-Mosquera, M. I., & Hornero-Méndez, D. (1994). Formation and transformation of pigments during the fruit ripening of *Capsicum annuum* cv. Bola and Agridulce. *Journal of Agricultural and Food Chemistry, 42*(1), 1555–1560. https://doi.org/10.1021/jf00043a006

Miranda-Dominguez, O., Ramirez, J. S. B., Mitchell, A. J., et al. (2022). Carotenoids improve the development of cerebral cortical networks in formula-fed infant macaques. *Scientific Reports, 12*, 15220. https://doi.org/10.1038/s41598-022-19279-1

Mortensen, A., & Skibsted, L. H. (1999). Carotenoid photobleaching. *Methods in Enzymology, 299*, 408–421. https://doi.org/10.1016/S0076-6879(99)99039-0

Mudalige, T., Qu, H., Van Haute, D., Ansar, S. M., Paredes, A., & Ingle, T. (2019). *Nanomaterials for food applications. In characterization of nanomaterials: Tools and challenges* (pp. 313–353). https://doi.org/10.1016/B978-0-12-814130-4.00011-7.

Murador, D. C., Salafia, F., Zoccali, M., Martins, P. L. G., Ferreira, A. G., Dugo, P., … Giuffrida, D. (2019). Green Extraction Approaches for Carotenoids and Esters: Characterization of Native Composition from Orange Peel. *Antioxidants, 8*(12), 613. https://doi.org/10.3390/antiox8120613

Pérez-Gálvez, A., & Mínguez-Mosquera, M. I. (2002). Lipid environment as modulator of the oxidation process of xanthophylls present in red pepper fruit (Capsicum annuum L.). In J. A. Empis (Ed.), *Functionalities of pigments in food* (pp. 315–318).

Qv, X. Y., Zeng, Z. P., & Jiang, J. G. (2011). Preparation of lutein microencapsulation by complex coacervation method and its physicochemical properties and stability. *Food Hydrocolloids, 25*(6), 1596–1603. https://doi.org/10.1016/j.foodhyd.2011.01.006

Ranard, K. M., Jeon, S., Mohn, E. S., Griffiths, J. C., Johnson, E. J., & Erdman, J. W., Jr. (2017). Dietary guidance for lutein: Consideration for intake recommendations is scientifically supported. *European Journal of Nutrition, 56*(Suppl. 3), 37–42. https://doi.org/10.1007/s00394-017-1580-2

Rodrigues, D. B., Mariutti, L. R. B., & Mercadante, A. Z. (2016). Two-step cleanup procedure for the identification of carotenoid esters by liquid chromatography atmospheric pressure chemical ionization-tandem mass spectrometry. *Journal of Chromatography A, 1457*, 116–124. https://doi.org/10.1016/j.chroma.2016.06.039

Rodrigues, D. B., Mercadante, A. Z., & Mariutti, L. R. B. (2019). Marigold carotenoids: Much more than lutein esters. *Food Research International, 119*, 653–664. https://doi.org/10.1016/j.foodres.2018.10.043

Sari, T. P., Mann, B., Kumar, R., Singh, R. R. B., Sharma, R., Bhardwaj, M., & Athira, S. (2015). Preparation and characterization of nanoemulsion encapsulating curcumin. *Food Hydrocolloids, 43*, 540–546. https://doi.org/10.1016/j.foodhyd.2014.07.011

Se Souza, C., Daood, H. G., Duah, S. A., Vinogradov, S., Palotás, G., Neményi, A., … Pék, Z. (2022). Stability of carotenoids, carotenoid esters, tocopherols and capsaicinoids in new chili pepper hybrids during natural and thermal drying. *LWT, 163*, Article 113520. https://doi.org/10.1016/j.lwt.2022.113520

Steiner, B. M., McClements, D. J., & Davidov-Pardo, G. (2018). Encapsulation systems for lutein: A review. *Trends in Food Science & Technology, 82*, 71–81. https://doi.org/10.1016/j.tifs.2018.10.003

Subagio, A., & Morita, N. (2003). Prooxidant activity of lutein and its dimyristate esters in corn triacylglyceride. *Food Chemistry, 81*(1), 97–102. https://doi.org/10.1016/S0308-8146(02)00384-9

Subagio, A., Wakaki, H., & Morita, N. (1999). Stability of lutein and its myristate esters. *Bioscience, Biotechnology, and Biochemistry, 63*, 1784–1786. https://doi.org/10.1271/bbb.63.1784

Teeranachaideekul, V., Boribalnukul, P., Morakul, B., & Junyaprasert, V. B. (2022). Influence of vegetable oils on in vitro performance of lutein-loaded lipid carriers for skin delivery: Nanostructured lipid carriers vs. Nanoemulsions. *Pharmaceutics, 14*(10), 2160. https://doi.org/10.3390/pharmaceutics14102160

Teo, S. Y., Lee, S. Y., Ong, H. L., Ong, C. L., Gan, S. N., Rathbone, M. J., & Coombes, A. G. A. (2015). Evaluation of biosourced alkyd nanoemulsions as drug carriers. *Journal of Nanomaterials, 2015*, 1–8. https://doi.org/10.1155/2015/537598

Tsao, R., Lee, S., Rice, P. J., Jensen, C., & Coats, J. R. (1995). Monoterpenoids and their synthetic derivatives as leads for new insect-control agents. In T. R. Baker, H. G. Cutler, & S. J. Stermitz (Eds.), *Synthesis and chemistry of agrochemicals IV, 584* pp. 312–324). American Chemical Society. https://doi.org/10.1021/bk-1995-0584.ch028.

Varma, K., Jude, S., Nair, R. V. R., Varghese, B. A., Jacob, J., Amalraj, A., & Kuttappan, S. (2021). Novel formulation of liposomal lutein using nanofiber weaving (NFW) technology: Antioxidant property and *in vitro* release studies. *Food Hydrocolloids for Health, 1*, Article 100025. https://doi.org/10.1016/j.fhfh.2021.100025

Wang, Y., Geng, M., Zhang, X., Yan, M., Sun, L., & Zhao, Q. (2023). Preparation of lutein nanoemulsion by ultrasonic homogenization method: Stability and *in vitro* anti-inflammatory activity. *Algal Research, 73*, Article 103154. https://doi.org/10.1016/j.algal.2023.103154

Weigel, F., Weiss, J., Decker, E. A., & McClements, D. J. (2018). Lutein-enriched emulsion-based delivery systems: Influence of emulsifiers and antioxidants on physical and chemical stability. *Food Chemistry, 242*, 395–403. https://doi.org/10.1016/j.foodchem.2017.09.060

Xavier, A. A. O., Mercadante, A. Z., Domingos, L. D., & Viotto, W. H. (2012). Desenvolvimento e validação de método espectrofotométrico para determinação de corante à base de luteína adicionado em iogurte desnatado. *Química Nova, 35*(10). https://doi.org/10.1590/S0100-40422012001000028

Yahia, E. M., & Ornelas-Paz, J. J. (2010). Chemistry, stability, and biological actions of carotenoids. In L. A. De La Rosa, E. Alvarez-Parrilla, & G. A. Gonzalez-Aguilar (Eds.), *Fruit and vegetable phytochemicals: Chemistry, nutritional value and stability* (pp. 177–222). Wiley-Blackwell.

Yang, S., Zhou, Q., Yang, L., Xue, Y., Xu, J., & Xue, C. (2015). Effect of thermal processing on astaxanthin and astaxanthin esters in Pacific white shrimp (*Litopenaeus vannamei*). *Journal of Oleo Science, 64*(3), 243–253. https://doi.org/10.5650/jos.ess14219

Young, J. C., Abdel-Aal, E.-S. M., Rabalski, I., & Blackwell, B. A. (2007). Identification of synthetic regioisomeric lutein esters and their quantification in a commercial lutein supplement. *Journal of Agricultural and Food Chemistry, 55*(13), 4965–4972. https://doi.org/10.1021/jf070357n

Zepka, L. Q., Borsarelli, C. D., Silva, M. A. A. P., & Mercadante, A. Z. (2009). Thermal degradation kinetics of carotenoids in a cashew apple juice model and its impact on the system color. *Journal of Agricultural and Food Chemistry, 57*(17), 7841–7845. https://doi.org/10.1021/jf900558a

Zhang, Y., Dawson, R., Kong, L., & Tan, L. (2024). Lutein supplementation for early-life health and development: Current knowledge, challenges, and implications. *Critical Reviews in Food Science and Nutrition*, 1–16. https://doi.org/10.1080/10408398.2024.2357275

Zhang, Y., Song, J., Wu, C., & Deng, G. (2025). Lutein-loaded lotus root starch nanoparticles: Preparation, release, and *in vitro* anti-inflammatory activity. *International Journal of Biological Macromolecules, 304*, Article 140785. https://doi.org/10.1016/j.ijbiomac.2025.140785