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Photo-stability of lutein in surfactant-free lutein-zein composite colloidal particles

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ABSTRACT

The ability of nanoparticles from the plant protein zein to protect lutein from light degradation was studied under various conditions. Lutein-zein nanoparticles were synthesized, after zein purification, by anti-solvent precipitation. Particle sizes, ranging from 25 to 75 nm, measured by dynamic light scattering, were tuned by varying zein concentrations in the solvent phase (before anti-solvent precipitation), which was linked to the encapsulation efficiency. However, changes in particle sizes did not result in significant changes in photo-stability. Zein-lutein nanoparticles showed increased photo-stability of lutein when compared to lutein dispersions in water. To further promote the lutein stability, ascorbic acid was used as an antioxidant in the aqueous dispersion. The addition of ascorbic acid to lutein-zein particles resulted in dispersions with similar properties. However, the photo-stability of lutein in dispersions stabilized with ascorbic acid improved significantly compared to samples without ascorbic acid or to pure lutein dispersions (about 25% increased relative stability).

1. Introduction

There are many types of natural colorants of which one group is the carotenoids. These are natural pigments that are present in fruits, vegetables, other plants, algae, and photosynthetic bacteria (Delgado-Vargas, Jiménez, & Paredes-López, 2000). Generally, carotenoids absorb wavelengths ranging from 400 to 550 nm (violet to green), which causes these compounds to be deeply colored yellow, orange, or red. These carotenoids can be roughly divided into two groups. The first consists of unoxygenated carotenoids such as α-carotene, β-carotene, and lycopene and are called carotenes. The second are oxygen containing carotenoids called xanthophylls, examples are lutein and zeaxanthin. The carotenoid used in this research is lutein (see Supplementary information Fig. S1a), a permitted food colorant (E161b). Lutein is a water insoluble component because of the presence of a long chromophore with conjugated double bonds. These bonds can easily be oxidized and degraded, which translates into a high sensitivity of lutein to light and heat. The sensitivity of lutein against ultraviolet and visible light originates from photochemical reactions due to this light. This leads to molecular excitation of susceptible compounds such as lutein. This molecular excitation is caused by photon energy, which is directly related to light wavelengths. When a ground state molecule is promoted to an excited state through light absorption, energy release can occur in several ways. Excited state molecules can release energy through heat, transfer of energy to other molecules, or emission of photons. Transfer of energy to other molecules can cause damage and may lead to off-flavor formation, color changes, and other negative sensory characteristics (Kline et al., 2011). Research suggests that lutein has two main roles: blue light filtration and an antioxidant function. In addition to lutein's use as a colorant, research indicates that lutein also has potential positive health benefits such as reducing the risk of eye diseases and improvement of cognitive functions (Eggersdorfer & Wyss, 2018), however additional research is needed (Ma & Lin, 2010).

Used as a colorant, lutein suffers from instability against light, heat, and oxygen, as do many natural (food) colorants (Sigurdson, Tang, & Giusti, 2017). Because of these deficiencies the demand for more stable pigments is raised by industry (Wissgott & Bortlik, 1996). A few solutions include patented processes to improve the stability of pigments by

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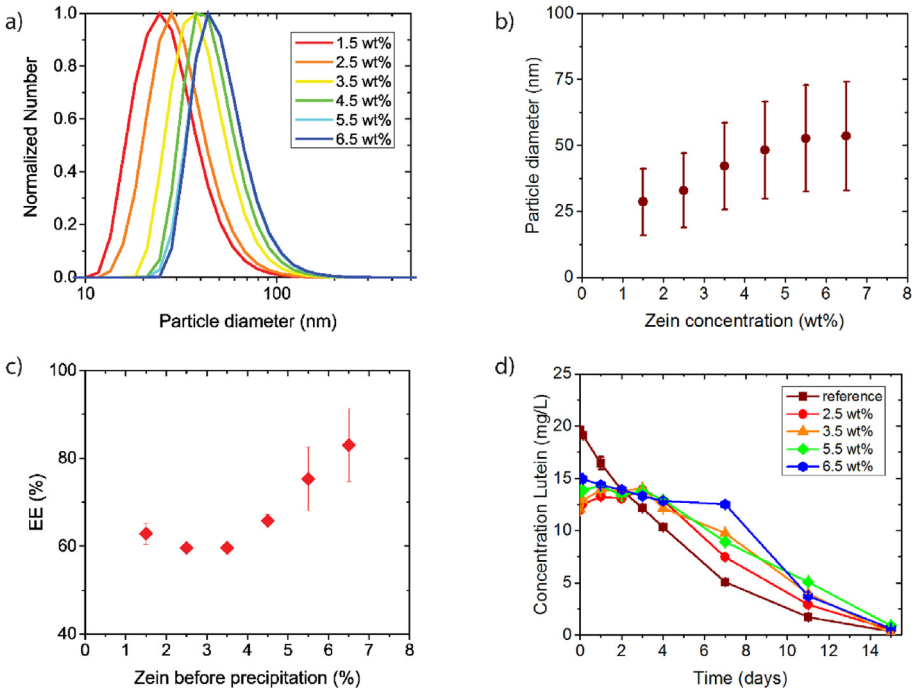

Fig. 1. Tuning the particle size of zein-lutein particles by increasing the zein concentration in the stock solution, while keeping the lutein concentration constant. (a) Shows the size distributions (number averaged) of the synthesized dispersions, (b) shows the average particle sizes with the standard deviations shown as error bars. Particle sizes were measured using dynamic light scattering. (c) Effect of increasing zein concentration at constant lutein concentration on the encapsulation efficiency. Experiments were performed in duplicate. (d) Effect of exposure to light on the concentration of lutein. UV–vis spectra are included in supplementary information Fig. S3. All particle dispersions were prepared using an initial amount of 0.010 wt% lutein (including the reference, which corresponds to 0.02 g/L).

chemical modification; however, such processes are not consistent with the label natural. A natural colorant must be stabilized using methods that are recognized as natural, such as encapsulation, if it is to continue to be termed natural (Wissgott & Bortlik, 1996). Encapsulation of sensitive natural pigments can offer a solution by embedding it in a protective matrix or by creating a barrier between the pigment and the environment (de Boer et al., 2019a,b; Steiner, McClements, & Davidov-Pardo, 2018). Additional benefits of encapsulation are that potential off-flavors may be masked by the biopolymer encapsulant (Yousuf et al., 2016) and that other ingredients such as stabilizers (examples include: sodium caseinate, Tween 80, Pluronic F68, Pluronic F127, Span 80 and lecithin) (Cheng & Jones, 2017; Farris, Brown, Ramer-Tait, & Pannier, 2017; Paliwal & Palakurthi, 2014; Podaralla & Perumal, 2012) can be co-encapsulated.

Lutein can be encapsulated using various methods (Steiner et al., 2018) which can be subdivided in two main methods: surfactant-based encapsulation systems or biopolymer-based encapsulation systems.

A surfactant-based encapsulation system was investigated in which stable lutein-enriched emulsions were prepared, stabilized by caseinate (Davidov-Pardo, Gumus, & McClements, 2016). Another research also describes surfactant-based system, using nanostructured lipid carriers (NLCs) (Lacatusu et al., 2013). These researchers conclude that NLCs have a better in vitro sustained release of lutein as compared to conventional nano-emulsions.

A biopolymer-based encapsulation system was investigated, in which complex coacervation method was used based on gelatin and gum Arabic (Qv, Zeng, & Jiang, 2011). The researchers conclude that microencapsulation is an adoptable method to protect lutein against light effects. Hu et al. also use a biopolymer-based encapsulation system, preparing nanoparticles using the plant protein zein using solution enhanced dispersion by supercritical fluids (Hu, Lin, Liu, Li, & Zhao, 2012). More recently, a simpler method was devised using liquid-liquid dispersion to prepare lutein-zein nanoparticles (Chuacharoen & Sabliov, 2016) using surfactants resulting in further improved stability against light. Other researchers even found that zein provides a degree of physical protection to encapsulated lutein in gastric conditions (Cheng, Ferruzzi, & Jones, 2019). In the above research, light stability experiments were performed, and surfactants were often added as stabilizers. However, the effect of an antioxidant on the light stability of lutein and the amount of light penetrating into the sample was often not taken into consideration. Therefore, in this paper, we investigate the encapsulation of lutein using the biopolymer zein, and we study the effect of an antioxidant (ascorbic acid, see Fig. S1b) on the light stability of lutein-zein particles.

Zein is a water-insoluble plant protein from corn. It has several beneficial properties for application in the food industry: it is edible, abundant, renewable, biodegradable and soluble in food safe solvents, such as water-ethanol mixtures (Lawton, 2002). The mechanism of formation of zein colloidal particles is well established (Patel & Velikov, 2014). Mostly, zein particles are synthesized using anti-solvent precipitation, often resulting in particles with a positive surface charge (Chatsisvili, Philipse, Loppinet, & Tromp, 2017). Thanks to these useful properties, zein nano-particles have been well studied for their application as a colloidal encapsulating agent focusing on the design of new food systems and functional ingredients such as food colorants; lipids; essential oils; flavors; anti-microbial agents; fat soluble vitamins; and natural anti-oxidants (Kasaai, 2018; Patel, 2018). Applications of these zein-based nano-sized materials resulted in products with a better quality, properties, functionalities, and a higher efficiency in comparison with the larger counterparts. Used as an encapsulating agent, zein is known to preserve the scavenging properties of antioxidants during manufacturing, storage, and distribution, and to enhance their physiological potency (Wu et al., 2012). When lutein is encapsulated by zein, the zein outer layer is expected to protect lutein from degradation by preserving the antioxidant properties of lutein and therefore keeping its yellow color, due to protein-mediated antioxidant effects of zein (Elias, Kellerby, & Decker, 2008) and when an antioxidant, such as ascorbic acid, is added then lutein is expected to degrade even slower.

In this work, particles were synthesized from purified zein using anti-solvent precipitation. The effect of the zein concentration in the synthesis on the lutein-zein particles was studied first. Then, ascorbic acid, also known as vitamin C (E300), was added to improve the stability of the lutein-zein particles. Ascorbic acid (Fig. 1b) is an antioxidant and forms the ascorbate anion when it is dissolved in water (Abbas, Da Wei, Hayat, & Xiaoming, 2012). Other research has confirmed the affinity between these anions and zein (Hatamie, Nassiri, Alivand, & Bhatnagar, 2018). Ascorbic acid is known to stabilize lutein upon illumination and elevated temperatures, due to its antioxidant

properties (Shi & Chen, 1997). Finally, the effect of ascorbic acid on the photochemical stability of the lutein-zein particles was investigated and compared to the light penetration depth of the dispersions.

2. Experimental

2.1. Materials

Food grade zein was purchased at Flo Chemical Corporation, type F4000C FG (lot nr. F40006021C2). Ethanol (absolute, technisolv) was purchased from VWR. L-Ascorbic acid (reagent grade), acetic acid and sodium acetate were purchased from Sigma-Aldrich and chloroform (HPLC grade, stab./amylene) from Biosolve. Food grade lutein (FloraGlo lutein, Crystalline dry) was kindly donated by DSM. Water was purified using a Millipore Direct-Q purification system.

2.2. Lutein-zein particle synthesis

Prior to particle synthesis, most of the colored impurities were extracted from the zein powder by washing this in ethanol (de Boer, Kok, Imhof, & Velikov, 2018) to eliminate color from the zein itself (Sessa & Woods, 2011). Then a zein stock solution in aqueous ethanol (70 wt%) was made. Lutein-zein nanoparticles were synthesized using anti-solvent precipitation, based on an earlier reported procedure (Chuacharoen & Sabliov, 2016). Briefly, 1 mL of 5 wt% purified zein solution in aqueous ethanol (70 vol%) was prepared. A lutein solution was prepared at 0.20 mg/mL with ethanol, where it is important to stay below the solubility limit (0.3 mg/mL) (Craft & Soares, 1992) of lutein in ethanol. This lutein solution was added dropwise to the zein solution at a volume ratio of 1:1 under light shaking. This was then added at once to 8 mL of milli-Q water under stirring (700 rpm). After preparation the sample was stirred for an additional 15 min. At this stage the samples are called “freshly-made”. To prepare lutein-zein particles in the presence of ascorbic acid (LZA), the lutein-zein solution was precipitated in 8 mL water including 0.20 mg/mL ascorbic acid.

Dialysis was used to remove the ethanol, using dialysis membranes with a molecular weight cut-off of 14,000 Da (Sigma-Aldrich). A two-day dialysis of the resulting dispersion against water adjusted to a pH of 4 with HCl was used and the medium was replaced 4 times. Concentrations were not adjusted after dialysis. The dispersion was collected and kept in the fridge (at 7 °C and in the dark) for further analysis.

Lutein dispersions without zein were prepared in parallel using the same method, with the exception that zein was not added.

2.3. Particle size, size distribution, and zeta-potential analysis

To determine size distributions, particle sizes were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS series (Malvern Instruments), which was also used to obtain zeta-potentials. In DLS, a CONTIN analysis was used to obtain the number averaged size distributions. To prevent multiple scattering, samples were diluted five times using a buffer of pH 4 (acetic acid and sodium acetate), to a suitable concentration prior to the measurements. At least three separate measurements were performed. The pH was measured using a Mettler Toledo FiveEasy pH meter.

2.4. Encapsulation efficiency (EE)

The unencapsulated lutein is finely dispersed in the medium and does not sediment upon centrifugation. Therefore, the encapsulation efficiency is determined by centrifugation of the zein-lutein particles. Here, we assume that a negligible amount of lutein is trapped by water pockets that might still be left in the pellet. To do this, 1 mL of the freshly-made Lutein-Zein particle dispersion was centrifuged at 21,000*g* for 3 h and 15 min. The supernatant and the nanoparticle pellet were separated using a pipette. Then the pellet was dissolved in 1 mL aqueous ethanol (90 vol%) overnight and kept in the dark. The next morning lutein was extracted with chloroform (1:1 ratio), after which the lutein was diluted two times with the chloroform-ethanol-water solution. The concentration of lutein was measured using a UV–Vis spectrophotometer (HP 8953A spectrophotometer, or Perkin Elmer lambda 365 UV–vis) with quartz cuvettes of 1 cm path length recorded at a wavelength of 452 nm. The absorbance value was converted to lutein concentration using an appropriate calibration curve using the same medium as the particle dispersions and Eq. (1) (Chuacharoen & Sabliov, 2016).

$$EE\,(\%) = \frac{c_{pellet}}{c_{initial}} 100\% \quad (1)$$

Here, $EE\,(\%)$ is the encapsulation efficiency, c_{pellet} is the concentration of lutein present in the pellet after centrifugation and $c_{initial}$ is the initial concentration of lutein that was added during the synthesis. The EE of all samples was measured within two days after synthesis.

2.5. Photo-chemical stability of lutein-zein particles

It has been investigated that the most damaging wavelengths for lutein stability are UV (200–400 nm) and 463 nm wavelengths (Kline et al., 2011). Therefore, we use light in the UV–Vis spectrum to test the photochemical stability of the colloidal dispersions in this research. Nanoparticle and reference samples (10 mL) were placed in transparent glass vials (FIOLAKS clear, Schott) and stored in a dark room in the light beam of a UV–Vis lamp (Siemens HBO 75 W – XBO 100 W, see Fig. S2 for its spectrum) for up to 20 days. A separate experiment was performed while placing the sample vials in the windowsill for several months (November until April). At exposure time intervals, 1 mL was withdrawn from each sample and then extracted as described in the preceding Section and analyzed for lutein concentration in the pellet using UV–vis spectroscopy (Perkin Elmer, lambda 365 UV–vis) at 452 nm. The experiments were performed in duplicate.

3. Results and discussion

3.1. Effect of zein concentration on lutein-zein particles

Zein-Lutein particles were synthesized using different amounts of zein while keeping the amount of lutein constant (see Table 1a). By increasing the concentration of zein, bigger particles could be synthesized (see Fig. 1a and b). This effect on the particle size was found to be statistically significant and was possible to obtain similar sizes when the same synthesis parameters were used, which corresponds to earlier

Table 1
(a) Tuning the particle size of zein-lutein particles by increasing the zein concentration in the stock solution, while keeping the lutein concentration constant. Concentrations zein and lutein in stock before precipitation. (b) Particle sizes of LZ and LZA dispersions measured at different time steps when illumination the samples using the Siemens HBO 75 W – XBO 100 W lamp (LZ = lutein-zein, LZA = lutein-zein-AA). The error is the standard deviation of the size distribution from DLS.

a)			b)		
Zein (wt %)	Lutein (g/L)	Lutein:Zein weight ratio	Illumination time (days)	LZ Particle diameter (nm)	LZA Particle diameter (nm)
1.5	0.10	0.0067	0	29.4 ± 14.4	24.0 ± 11.4
2.5	0.10	0.0040	7	42.8 ± 22.6	46.5 ± 24.1
3.5	0.10	0.0029	14	123.7 ± 62.6	48.2 ± 25.4
4.5	0.10	0.0022			
5.5	0.10	0.0018			
6.5	0.10	0.0015			

findings (de Boer et al., 2019a, 2019b; de Boer et al., 2018). Note that due to the relatively small particle sizes and low concentration of zein, the dispersions are nearly transparent but colored (see Section 3.4 for an example).

Interestingly, at low zein concentrations there seems to be a minimum encapsulation efficiency. Then, upon increasing the zein concentration, the encapsulation efficiency increased (Fig. 1c). This is according to expectations: since the amount of lutein did not change, there is more zein material available for encapsulation purposes and thus the encapsulation efficiency should increase.

Freshly made samples were used to determine the photochemical degradation of a selection of these lutein-zein particles with different zein wt% compared to a reference consisting of lutein in water. The samples were exposed to artificial light and analyzed spectrophotometrically at intervals for remaining lutein content, see Fig. 1d, and SI Fig. S3. Here, it is visible that the lutein concentration in the reference sample gradually decreased (Fig. 1d and S3a), which is according to expectations (Shi & Chen, 1997). The lutein-zein dispersions start at a lower concentration due to the different encapsulation efficiencies. For these lutein-zein dispersions, there first is a period of about 4 days during which the lutein content is constant. After that, the lutein concentration finally drops to a similar level as the reference sample without zein. This is also visible in the corresponding UV–vis spectra (Fig. S3). Due to the transparency of the samples, differences in scattering and light penetration do not explain the difference in degradation. Fig. 1d indicates that differences in lutein content at day 8, scaled with the zein content/size. However, this was just observed for only this day. Generally, it was observed that neither the amount of zein, nor the particle size makes a significant difference in the degradation profile.

3.2. The effect of ascorbic acid on the stability lutein-zein particles

Particle sizes and zeta potentials were measured after dialysis and after 7 days of storage, see Table 2. Here we can see that the particle sizes of the particles that were synthesized by precipitation in the presence of ascorbic acid (AA) are initially of similar size to the particles that were synthesized by precipitation in pure water. After having been stored for 7 days, a size increase was observed for samples that were synthesized by precipitation in ascorbic acid. This might be an indication of instability, however when measuring the zeta potentials of these samples no difference was observed. Interesting to note at this point is that compared to other research (Chuacharoen & Sabliov, 2016), smaller particle sizes and positive instead of negative zeta-potentials are obtained. The difference in zeta-potentials are explained by the fact that here a pH of 4 is used (Patel, Bouwens, & Velikov, 2010) while another research group used a pH of 7 (Chuacharoen & Sabliov, 2016). The apparent difference in particle sizes is an effect of the averaging that was chosen. Table 2 shows number averaged particle sizes, this was chosen since for polydisperse samples the number averaged data is comparable to SEM data. While the intensity averaged data, see SI Table S1, corresponds much more closely to previous studies (Chuacharoen & Sabliov, 2016).

Table 2
Particle sizes (number averaged) and zeta-potentials of Z, LZ, ZA, and LZA dispersions measured directly after dialysis, and measured after 7 days after dialysis (Z = zein, LZ = lutein-zein, ZA = zein-AA, LZA = lutein-zein-AA). All particle dispersions were prepared using an initial amount of 0.010 wt% lutein, 2.5 wt% zein and, for AA samples, 0.016 wt% of ascorbic acid. The error is the standard deviation of the size distribution from DLS or zeta-potential.

Sample	Particle diameter (nm) after dialysis	Particle diameter (nm) 7 days after dialysis	Zeta potential (mV) after dialysis	Zeta potential (mV) 7 days after dialysis
Z	38.1 ± 14.9	25.9 ± 12.1	27.5 ± 4.6	27.9 ± 4.1
LZ	41.8 ± 16.7	37.2 ± 14.9	28.9 ± 4.6	28.7 ± 4.9
ZA	24.7 ± 11.1	74.9 ± 43.0	27.4 ± 4.3	28.5 ± 4.3
LZA	34.8 ± 14.6	67.4 ± 37.5	29.4 ± 4.4	29.0 ± 4.7

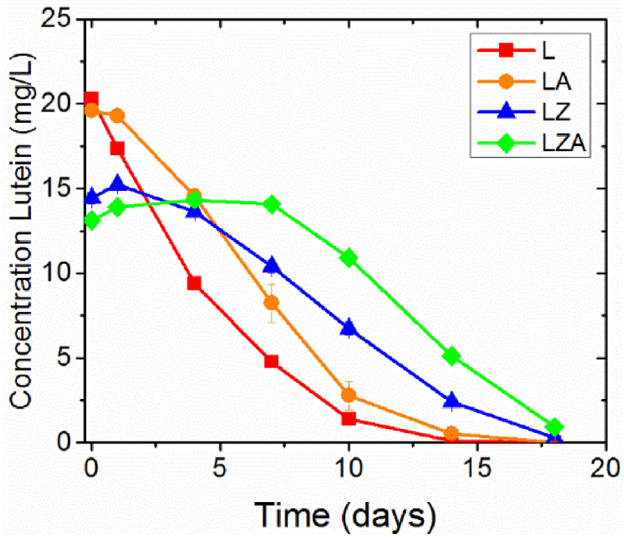

Fig. 2. Lutein content over time of lutein-zein particle dispersions and references placed in a dark room after illumination by a Siemens HBO 75 W – XBO 100 W lamp. Stability over time is shown for lutein (L) reference, lutein and AA (LA) reference, a dispersion with 2.5 wt% zein concentration and lutein (LZ), and a dispersion with 2.5 wt% zein concentration, lutein and AA (LZA).

3.3. Effect of ascorbic acid on the photochemical stability

Samples containing ascorbic acid were prepared according to Section 2.2, in which the milli-Q water, in which the lutein-zein mixture was precipitated, contained 0.20 mg/mL ascorbic acid. For these experiments only freshly made samples were used and reference samples were also made using this method. Following this, samples were illuminated by the UV–Vis lamp or by daylight, samples were taken at different time intervals, and the remaining lutein content inside of the particles was determined.

The degradation profiles of the samples illuminated by the lamp are represented in Fig. 2. Here it is visible that the reference samples (L and LA) show a gradual decrease in lutein content, which seems to be relatively similar and is comparable to earlier results (Section 3.1) as well as to literature (Shi & Chen, 1997). The presence of AA slows down the degradation slightly. This gradual decrease is also visible in the raw spectroscopy data (see SI Fig. S4a and b). The lutein-zein dispersion (LZ) and the lutein-zein-AA (LZA) dispersion both seem to have a plateau after which the lutein content decreases (see Fig. 2, and S4c and d). The plateau is longer for LZA, which indicates that the presence of ascorbic acid in combination with zein improves the stability of lutein the most. Overall, these experiments again show that encapsulation of lutein in zein increases its photo-stability. Also, ascorbic acid has a positive effect on the lutein photo-stability compared to the lutein-zein particles without the addition of ascorbic acid. This confirms our hypothesis.

Another batch of samples was synthesized and illuminated by daylight, of which the results are presented in Fig. 3 (corresponding UV–Vis spectra can be found in SI Fig. S5). Similar to previous results, the lutein reference (L) and the lutein reference in the presence of ascorbic acid (LA) seem to have similar degradation profiles. Note that the initial lutein content is different, however, the trend is the same (see also Fig. S4a and b, the small jump in signal in Fig. S5a and b is due to the changing of the source in the spectrophotometer at 400 nm). The lutein-zein dispersions (LZ and LZA) show a plateau before the concentration of lutein inside of the particles decreases, see also Fig. S5c and d for the spectroscopy data. For LZA the lutein concentration first increases and

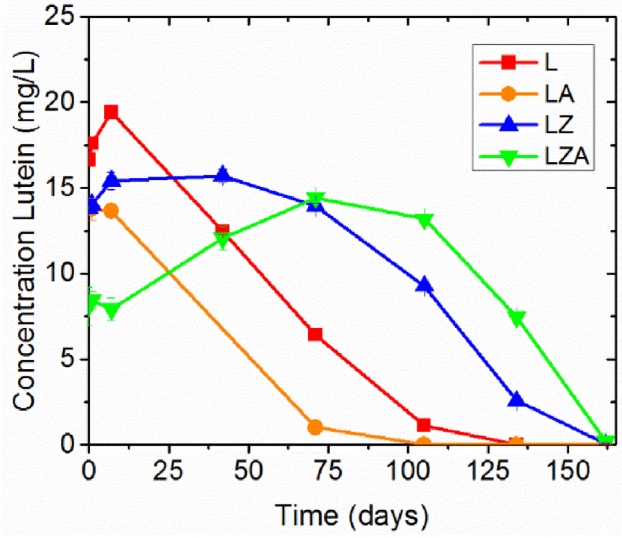

Fig. 3. Light stability over time of lutein-zein particle dispersions and references samples placed in the windowsill and illuminated by daylight (October until April). Stability over time is shown for the lutein (L) reference, lutein and AA (LA) reference, a dispersion with 2.5 wt% zein concentration and lutein (LZ), and a dispersion with 2.5 wt% zein concentration, lutein and AA (LZA).

later decreases (Fig. 3). One explanation is that during the centrifugation unencapsulated lutein could be physically trapped in water pockets in the pellet, which could explain also the increased encapsulation reading for LZ. However, a similar increased reading is observed for L and in this sample, there is no zein pellet in which water (and lutein) can be trapped. Another possibility is that zein particles are aggregating or flocculating over time due to light and temperature, after which lutein was released from the matrix.

Zein colloidal particles without steric stabilizers tend to aggregate when the pH is too close to the isoelectric point (~6.2) or when the ionic strength of the solution is too high (Pascoli, de Lima, & Fraceto, 2018; Patel & Velikov, 2014). This is because the electrostatic repulsion is then no longer sufficient to overcome van der Waals and hydrophobic attractions. Ascorbic acid can contribute to the aggregation of the zein particles by increasing ionic strength. This aggregation could also explain the increase in particle size over time (Dai, Zhou, Wei, Gao, & McClements, 2019), as further explained in Section 3.4.

3.4. Penetration depth and particle sizes upon illumination

After looking at the degradation profiles of lutein in lutein-zein particles and reference samples discussed in previous Sections, it is interesting to investigate the amount of light that reaches the lutein until it gets scattered or absorbed. One would expect for nearly transparent particle dispersions (see Fig. 4a) that all particles are exposed to the light and lutein degradation takes place throughout the whole dispersion. This is opposed to strongly absorbing or scattering dispersions where a large portion of the light has interacted with only the first few millimetres of the sample dispersion. The last case will have an unwanted averaging effect on the lutein content that is measured using the method described in Section 2.5, since we want to know the effect of encapsulation and addition of an antioxidant on the photo-stability of lutein and not the effect of turbidity.

For this purpose, the optical penetration depth is determined. Absorption and scattering cause the intensity of light to decay with depth at a rate determined by the material's extinction, τ. In general, τ is a function of wavelength and temperature, but for constant τ, intensity I decays exponentially with depth z according to the Lambert-Beer's law as: $I(z) = I_0 e^{-\tau z}$. In which I_0 is the incident intensity. This can be used to determine the optical penetration depth $\delta = 1/\tau$, which is the depth at which the intensity of the transmitted light drops to $1/e$ of its initial value (Sugioka & Meunier 2010). Penetration depths were determined by measuring direct transmission of which the results are presented in Fig. 4b.

As expected, not all wavelengths reach through the whole sample (of 1 cm) and the light gets absorbed or scattered, which explains the fact that L and LA have a faster lutein degradation than LZ and LZA. Interestingly, the results in Figs. 2 and 3 show that LZ has a faster degradation then LZA, despite it having a lower penetration depth. This indicates that ascorbic acid in combination with zein has a significant positive effect on the stability of lutein, more than ascorbic acid alone or just the encapsulation with zein.

During the illumination experiments it was noted that the samples including zein became more opaque over time. This effect can be observed in Fig. 1d, the penetration depth for LZ and LZA decreases in time, which indicates that particle sizes are increasing, and the dispersion is slowly aggregating or flocculating, see Table 1b. It seems that the stability of the zein colloids is affected during the experiment, causing the particle sizes to increase and the optical penetration depth to decrease for these samples. A possible cause is degradation of zein particles because of the light and temperature, since zein particle dispersions are known to be stable at low temperatures in the dark (Kasaai, 2018; Pascoli et al., 2018). In these experiments also degradation by bacteria cannot be excluded.

This information can be combined with that from the photo-stability experiments in Section 3.3, where it is observed that LZA dispersions lead to a slower lutein degradation than LZ dispersions. However, for LZA dispersions the penetration depth is constantly higher than for LZ dispersions. This is contradictory, since it would be expected that the sample with the lowest penetration depth has the highest lutein stability over time. These observations suggest that ascorbic acid has a strong effect on the stability of the particles and enhances the stability of the lutein. Possibly, by the flocculation and aggregation of the LZ particles lutein is released (Nieuwland, Papen-Bottenhuis, Drost, Slaghek, & Erich, 2016) which then degrades in the light without protection from either the zein or the ascorbic acid. Future experiments to prevent this aggregation and increase the stability could be in the direction of adding surfactants (Cheng & Jones, 2017; Chuacharoen & Sabliov, 2016; Dai et al., 2019; Pascoli et al., 2018) or crosslinking of the zein chains to form solid particles that are less eager to aggregate or flocculate (Chen, Xinsong, & Tangying, 2007; Elzoghby, El-Lakany, Helmy, Abu-Serie, & Elgindy, 2017). This could then result in a further increase in the lutein stability when this necessary for a specific application.

4. Conclusion

In this study, lutein-zein colloidal particles were successfully synthesized using anti-solvent precipitation, following initial purification of zein. Also, ascorbic acid was successfully added to the synthesis as an antioxidant. Particle sizes could be tuned by changing the zein concentration during synthesis. When particle sizes increased, the encapsulation efficiency also increased, which is according to expectations. Zein nanoparticles showed a significant ability to protect lutein from degradation compared to lutein dispersions in water. However, no significant influence of particle size on photochemical stability was observed during photo-stability experiments. To promote the stability of the entrapped colorant even further, ascorbic acid was used as an antioxidant. The addition of ascorbic acid to lutein and lutein-zein colloidal particles did not lead to an increase in the particle size or to any changes in the zeta-potential, compared to dispersions without ascorbic acid. However, the photo-stability of the nanoparticle's dispersions stabilized with ascorbic acid improved significantly compared to samples without ascorbic acid or lutein dispersions.

We have studied lutein and lutein-zein samples for a relatively short period of time (up to 20 days) using artificial illumination. In literature

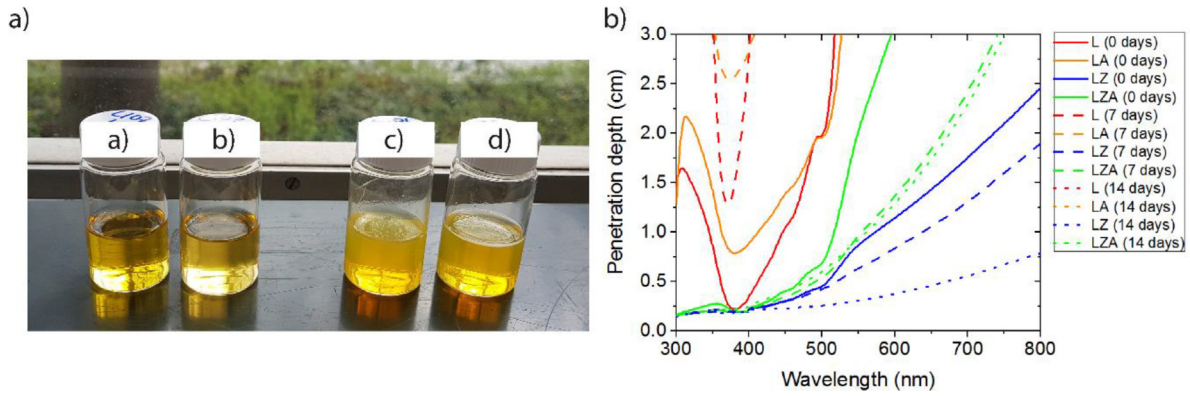

Fig. 4. (a) Freshly made lutein reference dispersions, (a.a) L and (a.b) LA, and lutein-zein dispersions, (a.c) LZ and (a.d) LZA. Due to the low concentration of zein not only the reference samples are colored but highly transparent, also the lutein-zein dispersions have a high transparency. (b) Optical penetration depth of L, LA, LZ and LZA dispersions measured at different time steps when illumination the samples using the Siemens HBO 75 W – XBO 100 W lamp (L = lutein, LA = Lutein-AA, LZ = lutein-zein, LZA = lutein-zein-AA).

studies with a longer illumination time are known for photo-stability measurements (Shi & Chen, 1997). Here, pure lutein + ascorbic acid was continuously illuminated at 4600 lx and 25 °C. Interestingly, the degradation was even faster for lutein + ascorbic acid (just several days) than observed in our studies. However, when alkaline conditions were used (by adding potassium hydroxide) the lutein content dropped to 65% at day 75. This would be an interesting follow up study.

These results show that the degradation rate of natural colorant lutein against photo-degradation can be significantly decreased by encapsulation using zein as carrier material. Addition of an antioxidant, like ascorbic acid, leads to an even further improved photo-stability of this colorant. The simple anti-solvent precipitation technique offers further possibilities to improve the stability, by adding surfactants, crosslinkers, and other stabilizers to ease the implementation of such particle systems into an application matrix when this is necessary for the specific application.

CRediT authorship contribution statement

Frankjen Ynske de Boer: Investigation, Writing - original draft, Visualization. **Arnout Imhof:** Conceptualization, Supervision, Writing - review & editing. **Krassimir Petkov Velikov:** Conceptualization, Supervision, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2019.100071.

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