



Thermal and irradiation resistance of folic acid encapsulated in zein ultrafine fibers or nanocapsules produced by electrospinning and electrospaying



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ABSTRACT

The objectives of this study were to characterize zein fibers and capsules prepared by electrospinning and electrospaying techniques, respectively, and then use them to encapsulate folic acid. Folic acid containing fibers and capsules (0.5, 1.0, and 1.5%, w/v) were submitted to thermal treatment (100, 140, and 180 °C) and ultraviolet A light (UVA) irradiation to evaluate the resistance of folic acid. Zein fibers and capsules containing folic acid showed high encapsulation efficiency (> 80%). Unencapsulated folic acid showed a reduction in folic acid content from 17.17 µg/mL to 5.44 µg/mL (approximately 70%) when exposed to 180 °C. Photodegradation of unencapsulated folic acid lowered its concentration from 17.17 µg/mL to 12.58 µg/mL (~26% of reduction), when exposed for 1 h to UVA irradiation. However, folic acid concentration when encapsulated in fibers (1.5%) was maintained or only slightly reduced from 9.73 µg/mg to 8.88 µg/mg after thermal treatment at 180 °C. The capsules containing 1.5% of folic acid also presented a slight reduction in folic acid concentration from 8.84 µg/mg to 7.88 µg/mg when exposed to 24 h of UVA irradiation. Zein fibers and capsules containing folic acid present promising characteristics for application in foods that require thermal processing or exposure to irradiation.

1. Introduction

Folic acid, also known as vitamin B9, is an essential component of a healthy diet. This micronutrient is the precursor of several enzymatic cofactors required for the synthesis of nucleic acids, amino acid interconversion, and DNA and RNA methylation reactions (Scott, Rebeill, & Fletcher, 2000). Folate deficit in the diet can lead to various disorders, such as congenital defects, cardiovascular disease, megaloblastic anemia, Alzheimer's disease, and cancers (Agrawal, Murphy, & Agrawal, 2007; Gazzali et al., 2016; Nukolova, Oberoi, Cohen, Kabanov, & Bronich, 2011).

Folic acid consists of an aromatic pteridine ring attached to *p*-aminobenzoic acid, which in turn is associated with one or more glutamic acids. When the chemical structure breaks down, the formed products are biologically inactive (Delchier, Herbig, Rychlik, & Renard, 2016). The chemical reactivity of folic acid makes this vitamin one of the most susceptible to losses during food storage and processing. Factors such as

temperature, light, pH, and the presence of catalysts or oxidizing agents contribute to the degradation of folic acid (Nguyen, Oey, Verlinde, Van Loey, & Hendrickx, 2003; Fukuwatari et al., 2009; Jastrebova et al., 2013).

Due to the low stability of folic acid, it is necessary to add high concentrations of this vitamin in the fortification of foods to ensure their health promotion effect (Acevedo-Fani, Soliva-Fortuny, & Martín-Belloso, 2018). Ingestion above recommended levels may lead to the appearance of unmetabolized folic acid in fetal and maternal circulation. It has been suggested that unmetabolized folic acid in maternal and fetal blood may act as a methyl donor for the regulation of gene expression, with consequences to fetal formation (Silva, Keating, & Pinto, 2017). Considering the importance of folic acid to human health, its vulnerability to external agents, and the potential risks associated with overeating, folic acid encapsulation may provide an alternative to its degradation in food products and an increase in its bioaccessibility. The encapsulation process may increase the bioaccessibility of folic

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acid, making it available for absorption in the small intestine. Thus, it is suggested that encapsulation also promotes increased bioavailability, as it is one of the most available forms for metabolization and distribution of physical activity.

Electrospinning and electro spraying techniques are versatile methods to encapsulate compounds, with a number of advantages when compared to traditional encapsulation techniques such as spray drying, coacervation, or ionic gelation. Among their advantages are the non-use of high temperatures, the high surface area and small diameter of the resultant fibers and capsules, and the high encapsulation efficiency (Aceituno-Medina, Mendoza, Lagaron, & Lopez-Rubio, 2015; Bhushani & Anandharamakrishnan, 2014; Zussman, 2011). The difference between electrospinning and electro spraying techniques is based on the concentration of the polymer solution. When the concentration of the solution is high (electrospinning), the jet exiting the capillary is in the form of a fiber and performs a whip-like motion until it reaches the collector plate. The solvent is evaporated during the process, resulting in the deposition of a solid nanofiber under the grounded bulkhead (Jaworek & Sobczyk, 2008). When the concentration of the polymer solution is low (electro spraying), droplets are formed. These highly charged droplets are self-dispersing and evaporation of the solvent leads to solidification, resulting in nanoparticles and nano-clusters on the collector.

The use of proteins for the micro- and nano-encapsulation of bioactive compounds has the advantage of non-toxicity for application in food and drugs (Ariyaratna & Karunaratne, 2015). Zein, the main storage protein of maize, is considered a safe food ingredient by the Food and Drug Administration (FDA); however, due to its hydrophobic character and its deficiency in amino acids (such as lysine and tryptophan), the use of corn protein in food products is limited. Zein is used in the manufacture of a wide variety of products, including textile fibers (Selling et al., 2007), biodegradable films, and packaging (Padgett, Han, & Dawson, 1998), and for the encapsulation of food and pharmaceutical products (Guo, Heinamaki, & Yliruusi, 2008).

Zein nanofibers produced by electrospinning showed an increased stability and bioavailability of gallic acid (Neo et al., 2013), β -carotene (Fernandez, Torres-Giner, & Lagaron, 2009), curcumin (Brahatheswaran et al., 2012), α -tocopherol (Saowakon & Suanantha, 2014), ferulic acid (Yang, Zha, Yu, & Liu, 2013), and tannins (Mori et al., 2014). Torres-Giner, Martinez-Abad, Ocio, and Lagaron (2010) produced zein capsules by an electro spraying process with docosahexaenoic acid (DHA, Omega-3) and reported the delay of oxidation reactions and an increase in chemical stability of the DHA. Peñalva et al. (2015), in a study using zein to encapsulate folic acid by spray-drying for pharmaceutical applications, evaluated the ability of zein nanoparticles to improve the bioavailability of folic acid. However, there are no studies on the evaluation of the thermal resistance of folic acid encapsulated with zein by electro spraying or electrospinning at temperatures simulating the processing of food for application in the food industry. In addition, research is needed to evaluate the exposure of encapsulated folic acid to different levels of ultraviolet irradiation.

Since encapsulation could improve the resistance of folic acid, the objective of this work was to evaluate the resistance of encapsulated folic acid in zein ultrafine fibers or capsules produced by the technique of electrospinning/electro spraying to thermal and UVA irradiation treatments.

2. Materials and methods

2.1. Materials

Folic acid and zein were purchased from Sigma-Aldrich. The other reagents were of analytical grade.

2.2. Preparation of the polymer solution

Polymer solutions of pure zein and zein with folic acid were used to prepare ultrafine fibers by electrospinning and capsules by electro spraying. Polymer solutions were prepared using 30% (w/v) zein for fiber formation and 9% (w/v) zein for capsule formation in 70% (v/v) ethanol solution containing sodium carbonate (0.01 mol L^{-1}). The polymer solutions were stirred using a magnetic stirrer for 2 h. Polymeric solutions of zein and folic acid were prepared under the same conditions, but with the addition of folic acid at concentrations of 0.5, 1.0, and 1.5% (w/w). The concentrations of zein and folic acid were determined based on preliminary tests to obtain fibers or capsules with uniform morphology.

2.3. Characterization of polymer solutions

The polymer solutions were characterized by apparent viscosity and electrical conductivity. The apparent viscosity was determined in a Brookfield digital viscometer with a spindle (No. 18, model DV-II, USA). The electrical conductivity was evaluated with a conductivity meter (Tecnom model - mCA-150P), and expressed in $\mu\text{S/cm}$. The evaluations were performed at room temperature ($\sim 23^\circ\text{C}$) using a 20 mL sample for each analysis.

2.4. Production of fibers/capsules by electrospinning/electro spraying

The equipment used to make the fibers and capsules, located inside a closed chamber, consisted of a high voltage source ($-30/+30 \text{ kV}$, Instor, England), an infusion pump (kdScientific, Model 200, England), and a fixed collector, mounted horizontally. Each polymer solution was individually placed in a 1 mL syringe with a 0.7 mm diameter stainless steel outlet port. For fiber and capsule production, the feed rates used were 1.0 mL/h and 0.6 mL/h, respectively. The electrospinning/electro spraying process was conducted by connecting the positive electrode ($+16 \text{ kV}$) from the DC power source (INSTOR, INSES-HV30, Brazil) to the needle. A negative electrode was connected to a stainless steel collector that was covered with aluminum foil and positioned horizontally to the infusion pump for deposition of the material. The distance from the tip of the needle to the collecting target was 16 cm for fiber formation and 10 cm for capsule formation. The process was controlled at $23 \pm 2^\circ\text{C}$ by an air conditioner and the relative humidity was set at $45 \pm 2\%$ using a dehumidifier.

2.5. Fiber and capsule morphology and diameters

Fibers and capsule morphology was evaluated by scanning electron microscopy (SEM) (Jeol, JSM-6610LV, USA). A small portion of the material was fixed in stubs using a double-sided tape and covered with gold using a metallizer (Sputtering, Deston Vacuum Deskv, USA). Images were captured under an acceleration voltage of 10 kV with a magnification of 1000 and $10,000\times$ for the fibers and 5000 and $30,000\times$ for the capsules. Mean diameter was determined from the calculated average of 50 fibers/capsules obtained from the SEM images, using the ImageJ computer program.

2.6. Functional groups

Functional groups and interactions of unencapsulated and encapsulated folic acid in the form of zein fibers and capsules were evaluated on a Fourier Transform Infrared (FTIR) spectrometer (Shimadzu, IRAffinity, Japan) with an ATR (Attenuated Total Reflectance) accessory. Scans were performed in the spectral range of 800 to 4000 cm^{-1} , and 32 readings were collected at a resolution of 2 cm^{-1} . The signals were processed using IRsolution software.

2.7. Thermogravimetric analysis (TGA)

Thermogravimetric analysis of unencapsulated and encapsulated folic acid in the form of fibers and capsules was carried out according to the method of Ariyaratna and Karunaratne (2015) by a thermogravimetric analyzer (TGA, TA-60WS, Shimadzu, Kyoto, Japan). Samples (approximately 5 mg) were heated in platinum capsules in the range of 30 to 600 °C, with a heating rate of 10 °C min⁻¹ and a nitrogen flow of 50 mL min⁻¹. An empty platinum capsule was used as a reference.

2.8. Encapsulation efficiency

The encapsulation efficiency of folic acid in the zein fibers or capsules was assessed by determining the total folic acid present in the material and the content of encapsulated folic acid. The sample (10 mg) was dissolved in 5 to 15 mL of 70% ethanol and 20 µL sodium hydroxide (1 mol L⁻¹), then vortexed for 2 min; and for complete solubilization, the samples were sonicated for 5 min. The material was centrifuged for 5 min at 2935 × g and filtered using a nylon membrane (0.22 µm). To determine the content of encapsulated folic acid, a rinse with ultrapure water was first performed to remove folic acid that had not been encapsulated, and then the material was vortexed and sonicated as described above. These materials were analyzed by high performance liquid chromatography (HPLC; Shimadzu, Japan) coupled to a high-resolution quadrupole-time-of-flight mass spectrometer (MS-QToF, Bruker Daltonics, Bremen, Germany). For chromatographic separation a Diol-75 (100 mm × 2.1 mm) column (MicroSolv Technology Corporation, Leland, NC, USA) was used. The mobile phases were water acidified with 0.1% formic acid (eluent A) and acetonitrile acidified with 0.1% formic acid (eluent B). The flow rate was 0.2 mL min⁻¹ and the column temperature was maintained at 40 °C. The mass spectrometer was operated in the ESI positive mode, with spectra acquired over a mass range of *m/z* 50 to 1200. MS parameters were: 4 kV capillary voltage, nebulizing gas pressure (N₂) of 2 bars, drying gas at 8 L min⁻¹, source temperature of 180 °C, and a RF collision of 150 Vpp; then 70 mS was transferred, with a pre-pulse storage of 5 mS. The equipment was calibrated with 10 mM sodium formate, covering the entire acquisition range (*m/z* 50 to 1200). In addition, automatic MS/MS experiments were performed by adjusting the collision energy values as follows: *m/z* 100, 15 eV; *m/z* 500, 35 eV; *m/z* 1000, 50 eV, using nitrogen as the collision gas. The encapsulation efficiency (E) was calculated according to Eq. 1.

$$E (\%) = \frac{\text{Encapsulated Folic Acid Content}}{\text{Total Folic Acid Content}} \times 100 \quad (1)$$

2.9. Thermal and UVA irradiation resistance

The resistance of unencapsulated and encapsulated folic acid in the fiber or capsule form was evaluated at temperatures of 100, 140, and 180 °C for 45 min; and under exposure to UVA irradiation (λ 365 nm) for 1, 12, and 24 h. After the heating or irradiation exposure, 10 mg samples containing 0.5, 1.0, or 1.5% (w/w) of fibers or capsules, were diluted in 5, 10 or 15 mL of ethanol (70%; v/v), respectively, and homogenized in a vortex for 2 min. For complete solubilization, the samples were sonicated for 5 min. The material was centrifuged for 5 min at 2395 × g and filtered using a nylon membrane (0.22 µm). The sample of 10 mg of unencapsulated folic acid was first diluted in 10 mL of ethanol (70%; v/v) and an aliquot of this solution was diluted 125 × for calibration. Folic acid was analyzed by liquid chromatography - mass spectrometry (LC-MS), as described in item 2.8. The amounts of folic acid encapsulated were expressed in µg/mg of fibers or capsules and the unencapsulated folic acid in µg/mL of ethanol.

The products of degradation of unencapsulated folic acid, exposed at 180 °C or 24 h of UVA irradiation, were also evaluated by LC-MS. MS data were analyzed using Data Analysis 4.0 software (Bruker Daltonics,

Bremen, Germany). The identification was performed by comparing the high-resolution *m/z* and MSⁿ fragmentation patterns with data from databases (METLIN, KEGG compounds, PubChem, Mass Bank, Maven, FoodB, and ReSpect) and reference literature with a mass accuracy window of 5 ppm.

2.10. Statistical analysis

Analytical determinations for the samples were performed in triplicate and standard deviations were reported. All data was subjected to an analysis of variance (ANOVA) and means of electrical conductivity and viscosity for different zein solutions with varying folic acid concentrations (0, 0.5, 1.0, and 1.5%; w/w) and means for encapsulation efficiency, thermal resistance, irradiation resistance of encapsulation, and percentage of folic acid were compared by Tukey's test (*p* < .05).

3. Results and discussion

3.1. Characterization of polymer solutions

The electrical conductivity and apparent viscosity of zein solutions with different concentrations of folic acid are shown in Table 1. The electrical conductivity of the solution is influenced by the type and concentration of the polymer, the solvent, and the availability of ionizable compounds (Bhardwaj & Kundu, 2010). The electrical conductivity values of the solutions with 30% (w/v) zein were higher than those of the 9% (w/v) solutions of the polymer (Table 1). The higher electrical conductivity in the solutions with a higher concentration of zein is associated with an increase of ionizable groups in solution. There are also electrostatic hydrogen bond interactions between the NH of the zein and the CO of folic acid. In both, 30% and 9% zein solutions, the addition of folic acid reduced the electrical conductivity. However, this reduction did not affect the formation of fibers and capsules, since there was a continuous and stable jet formation allowing the production of continuous fibers and capsules, indicating that the incorporation of the vitamin did not negatively affect the electrospinning/electrospraying process.

The polymer solution with 9% (w/v) zein had a very low viscosity and it was not possible to determine the apparent viscosity with the equipment available. The addition of folic acid in the 30% zein polymer solution increased its apparent viscosity, suggesting an interaction between folic acid and the protein. According to Aytac, Kusku, Durgun, and Uya (2016), this increase in the viscosity is due to a greater entanglement of polymer chains in solution. The increase in viscosity and the reduction in electrical conductivity of the polymer solutions containing folic acid did not influence the process of fiber and capsule

Table 1

Apparent viscosity and electrical conductivity of 30% and 9% zein solutions with different concentrations of folic acid.

Polymer solution ^a		Electric conductivity (µS/cm)	Viscosity apparent (cP)
Zein (%; m/v)	Folic acid (%; m/v)		
30	0.0	1330.0 ± 4.3 ^a	104.9 ± 6.3 ^d
30	0.5	1255.7 ± 4.5 ^b	121.3 ± 3.4 ^c
30	1.0	1201.7 ± 5.9 ^c	133.3 ± 3.2 ^b
30	1.5	1158.0 ± 4.3 ^d	143.3 ± 2.0 ^a
9	0.0	1046.7 ± 2.1 ^a	n.d
9	0.5	1041.3 ± 1.2 ^b	n.d
9	1.0	995.3 ± 1.6 ^c	n.d
9	1.5	988.0 ± 0.5 ^d	n.d

^a Different letters among the same concentration of zein solutions in the same column represent a significant difference among the averages submitted to the Tukey test at 5% of significance. n.d: not detected.

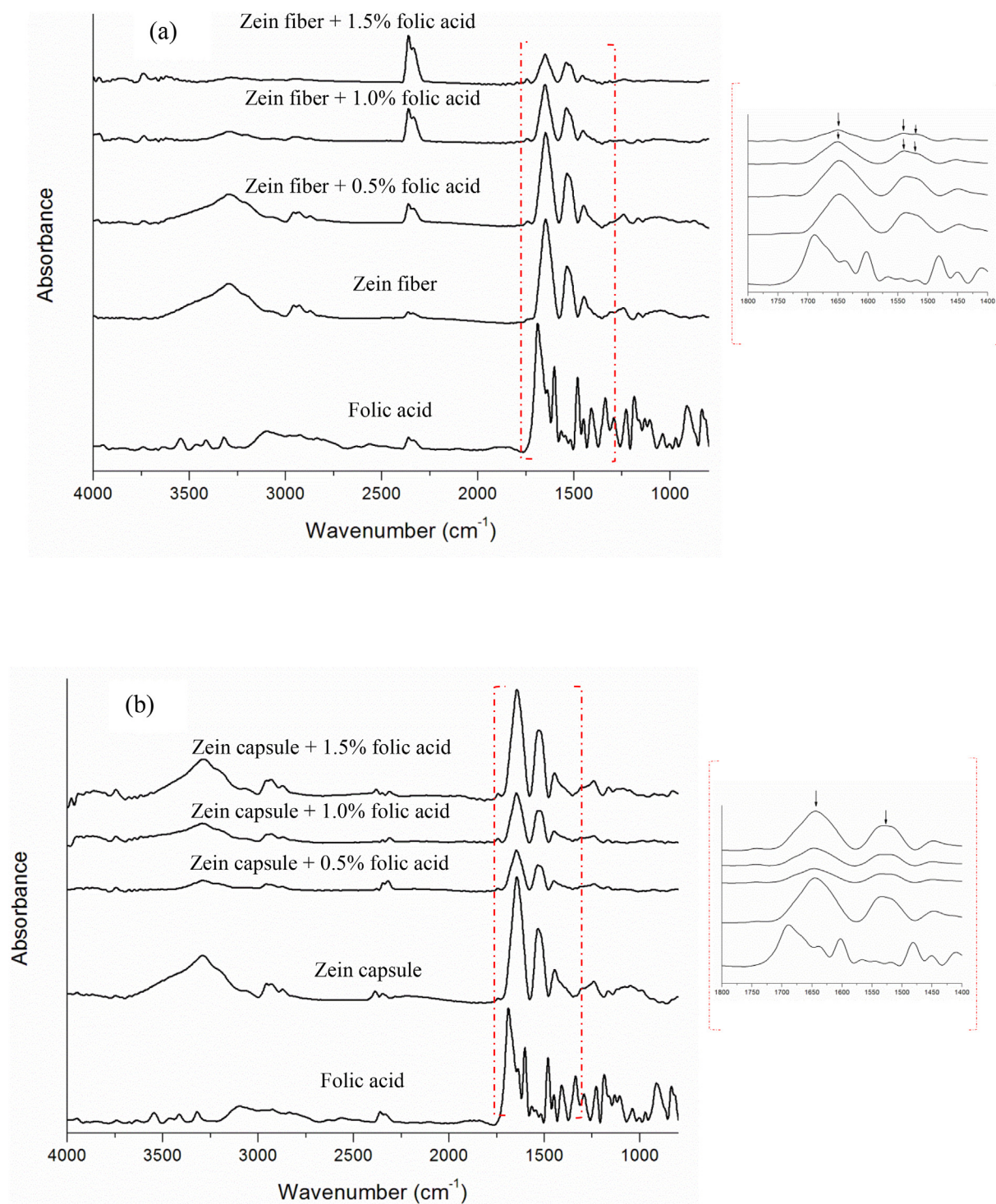


Fig. 1. FTIR-ATR spectra of unencapsulated pure folic acid, pure zein fibers, zein fibers with 0.5% folic acid, zein fibers with 1.0% folic acid and zein fibers with 1.5% of folic (a); and zein capsules with 0.5% folic acid, zein capsules with 1.0% folic acid and zein capsules with 1.5% folic acid (b).

formation; however, it caused an increase in their average diameter. In general, a higher viscosity and lower electrical conductivity result in a smaller jet stretch of the solution, thus producing thicker fibers (Wen et al., 2016). Pérez-Masiá et al. (2015) observed similar results, including increased viscosity and reduced electrical conductivity, by adding folic acid to milk protein solution.

3.2. Functional groups

FTIR analysis was performed to evaluate the interactions between

zein and folic acid (Fig. 1). The spectrum of unencapsulated pure folic acid is characterized by absorption bands in the regions of 4000 cm⁻¹ and ~900 cm⁻¹ (Fig. 1a and b). Bands characteristic of C=O deformation are observed at ~1695 cm⁻¹, while the overlapped band (~1607 cm⁻¹) refers to N–H vibration. The band 1481 cm⁻¹ is attributed to the vibration of phenyl ring and of pteridine. The folic acid bands observed in this study are in agreement with the work of Aceituno-Medina et al. (2015) and Assadpour, Jafari, and Maghsoudlou.Y. (2017).

Pure zein fibers and capsules show amide I and II bands identified at

$\sim 1643\text{ cm}^{-1}$ and $\sim 1530\text{ cm}^{-1}$, respectively (Fig. 1a and b). The amide I band is related to C=O bond stretching and the amide II band is associated with the symmetrical deformation of the N–H bond. However, the bands in the 3995 to 2872 cm^{-1} region refer to an O–H stretch superimposed on the N–H stretch and asymmetric and symmetric C–H stretches relative to the free fatty acid derivatives present in zein (Dashdorj et al., 2015).

The encapsulation of 1.0 and 1.5% folic acid by zein fibers promoted a displacement of the amide I band, since the 1643 cm^{-1} band in the pure zein fibers shifted to 1651 cm^{-1} in the fibers containing folic acid, for both concentrations. There is an overlap of the characteristic band of amide II folic acid observed as a small shoulder at 1515 cm^{-1} , and the band for zein fibers at 1539 cm^{-1} (Fig. 1a). In these spectra, the disappearance of the characteristic O–H stretch band (~ 3000 – 3600 cm^{-1}) is observed, which suggests the formation of hydrogen interactions between components of the fiber (Chen, Xiumei, & Fengling, 2007). Aceituno-Medina et al. (2015) reported similar spectra for fibers developed with folic acid, amaranth protein, and pullulan.

FTIR-ATR spectra for the pure zein and folic acid-containing zein capsules are shown in Fig. 1b. In general, there was no modification of the protein secondary structure, since no spectral band shifts are observed for 0.5 and 1.0% folic acid capsules. However, for capsules with 1.5% folic acid, there was a displacement in the amide I and II bands, since they are observed in the pure zein capsules at 1643 cm^{-1} and 1530 cm^{-1} , and in the capsules containing folic acid these are shifted to 1642 cm^{-1} and 1523 cm^{-1} , respectively. Some studies have reported that folic acid is able to conjugate with different polymers through an interaction of the amide II (NH) with the carboxylic group of the bioactive (C=O), which can be evidenced by the displacement observed for the capsules containing 1.5% folic acid (Sudimack & Lee, 2000; Teng et al., 2013).

Interaction of the polymer with the vitamin is important for improving its resistance to different conditions of exposure with variations in processing, as well as after its consumption. Zein nanoparticles help protect the folic acid from the gastric environment, where free folic acid can precipitate, and act as folic acid carriers for the absorptive membrane of the intestine. The properties of zein would be responsible for an increase in the lifetime of folic acid in the upper gastrointestinal tract, where its absorption is favored (Peñalva et al., 2015).

3.3. Morphology and size distribution of fiber/capsule diameter

Zein fibers and capsules containing 0.0%, 0.5%, 1.0%, and 1.5% of folic acid were observed by SEM to characterize their morphology and average diameters (Figs. 2 and 3). The conditions used in processing allowed the formation of fibers and smooth particles with homogeneous and continuous formats. The addition of folic acid did not affect the morphology of the fibers and capsules; however, the mean diameter in both treatments was altered. This can be attributed to the increased viscosity of the solutions when incorporating folic acid (Table 1). The mean diameter of fibers ranged from 369 nm for zein-only fibers (Figs. 2a–b) to 702 nm for fibers with zein and 1.5% folic acid (Figs. 2g–h). For capsules, those containing 0.5% of folic acid had the lowest mean diameter of 268 nm (Figs. 3c–d), while those containing 1.0% of folic acid had the largest mean diameter of 407 nm (Figs. 3e–f). Altan, Aytac, and Uyar (2018) also used 30% zein solutions and reported a mean diameter of pure zein fibers at 604 nm, whereas zein fibers containing 5, 10, and 20% carvacrol, presented diameters of 647, 539, and 553 nm, respectively. Baspinar, Ustundas, Bayraktar, and Sezgin (2018) used 5% zein solution for the encapsulation of curcumin by electro-spraying and observed a range of capsule diameters between 300 and 600 nm.

3.4. Thermogravimetric analysis (TGA)

TGA curves are shown in Fig. 4 of unencapsulated and encapsulated

folic acid in the form of zein fibers and capsules containing 0.5, 1.0, and 1.5% (w/v) folic acid. The thermogravimetric analysis was performed to observe if there was thermal protection of folic acid in zein fibers and capsules. Pure folic acid showed a water loss at about 125°C and its decomposition temperature was 257.73°C . The decomposition temperature of folic acid in the fibers varied from 305 to 313°C , whereas for the capsules this ranged from 298 to 303°C (Fig. 4a). Therefore, the incorporation of folic acid in the form of zein fibers and capsules, produced by electrospinning/electrospraying, contributes to an increase of the thermal resistance of the vitamin. These results suggest that the encapsulated form can be used in thermal processing, such as bakery products, which use high temperatures. Aceituno-Medina et al. (2015) also reported an increase in the thermal resistance of folic acid in fibers from amaranth protein isolate and pullulan. Ariyaratna and Karunaratne (2015) reported a decomposition temperature of 293°C for folic acid encapsulated in chickpea protein using the freeze-drying process.

3.5. Encapsulation efficiency and thermal and UVA irradiation resistance of folic acid

Since folic acid powder is commonly added in wheat and corn flours for fortification purposes, the resistance of encapsulated folic acid was evaluated while heating in an oven for 45 min at temperatures of 100, 140, and 180°C . Folic acid-fortified flours are consumed after thermal processing, so the thermal resistance evaluation conditions were chosen to simulate the baking processes of breads and cakes.

The unencapsulated folic acid exposed at 140°C and 180°C showed reductions of approximately 11% and 70%, respectively, after thermal treatment (Table 2). Based on thermogravimetric analysis (Fig. 4), the decomposition temperature of folic acid is 257.73°C ; however, Vora et al. (2002) reported that at 180°C , the breakdown of glutamic acid bonds occurs, and therefore the active function of the vitamin is lost.

Table 2 shows that thermal treatments at 100, 140, and 180°C did not influence the content of folic acid encapsulated in zein fibers at the 0.5% and 1.0% levels when compared to fibers without exposure to the heat treatment, indicating that there was thermal protection of the vitamin. A slight reduction in thermal resistance was seen in fibers containing 1.5% folic acid. The capsules with 0.5% of folic acid exposed to all the thermal treatments presented similar behavior to the capsules without exposure to high temperature, while the capsules with 1.0% and 1.5% of folic acid showed a slight decrease in folic acid content (Table 2). Ariyaratna and Karunaratne (2015) encapsulated folic acid in chickpea protein by freeze-drying, and evaluated the thermal degradation at 80, 120, and 160°C for 30 min. They reported thermal degradations of 18.3%, 29.0%, and 33.8%, respectively, for encapsulated folic acid; whereas for unencapsulated folic acid, there was thermal degradation of 25.9%, 42.7%, and 52.9%, respectively.

The resistance of the unencapsulated and encapsulated folic acid in the form of zein fibers and capsules was also evaluated by exposure to UVA irradiation for 1, 12, or 24 h (Table 3). Unencapsulated folic acid exposed to UVA irradiation for 1 h showed a reduction of approximately 26% compared to folic acid without exposure to UVA irradiation.

Zein fibers containing 0.5, 1.0, and 1.5% of folic acid, when exposed for 1 or 24 h of UVA irradiation, showed resistance comparable to fibers not exposed to irradiation (Table 3). The fibers exposed for 12 and 24 h of irradiation had a small reduction in vitamin resistance. The zein capsules, in general, showed resistance to UVA irradiation, with the exception of the capsule containing 1.0% of folic acid, exposed to irradiation for 12 h, where a reduction in resistance of the compound was observed. The loss of vitamins, even when protected in the zein matrix, is very small however, when compared to that of the unencapsulated folic acid. Thus, protection of the compound by encapsulation using electrospinning and electro-spraying was satisfactory.

According to Juzeniene, Tam, Iani, and Moa (2013), folic acid is

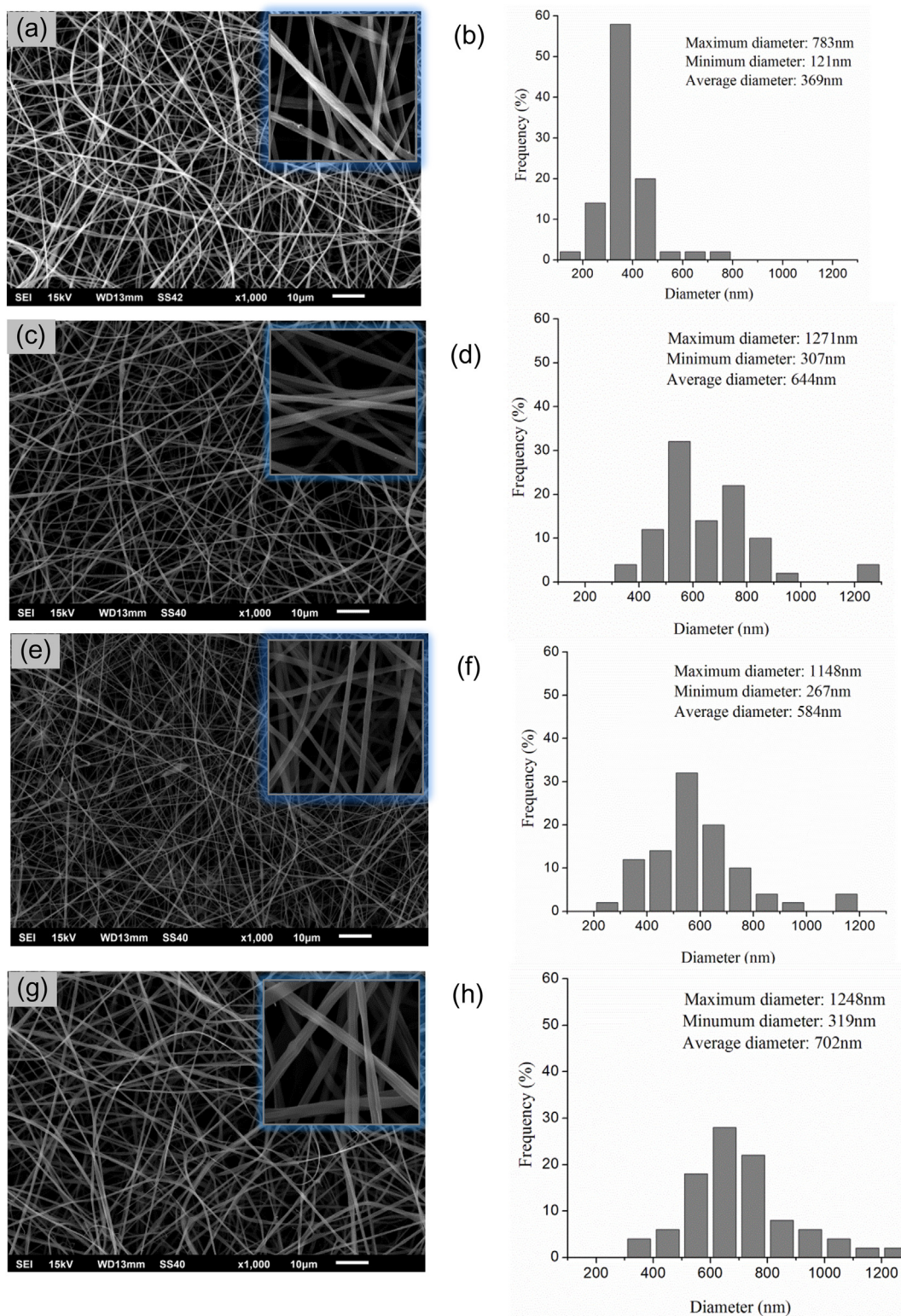


Fig. 2. Morphology of ultrafine fibers and respective size distribution: pure zein (a, b), zein with 0.5% folic acid (c, d), zein with 1.0% folic acid (e, f) and zein with 1.5% folic acid (g, h). Image with amplitude of 10,000 × in the upper right corner of the SEM.

sensitive to UV irradiation due to excitation of the bonding between C9 and N10, leading to its cleavage and the formation of *p*-aminobenzoyl-L-glutamic acid, 6-formylpterin or 6-carboxypterin as photodegradation products. Zein is characterized by the predominance of hydrophobic amino acids such as proline, isoleucine, alanine, phenylalanine, methionine, valine, and leucine; while folic acid is a hydrophilic

compound (Prasanna, Vasal, Kassahun, & Singh, 2001). The resistance of folic acid in both fibers and capsules may be related to the interaction of the vitamin with these amino acids, which makes it difficult to cleave the C9-N10 bonding of folic acid. Perez-Maziá et al. (2015) encapsulated folic acid (1.5% w/v) in whey protein and resistant starch by electrospraying, and evaluated its photodegradation using natural

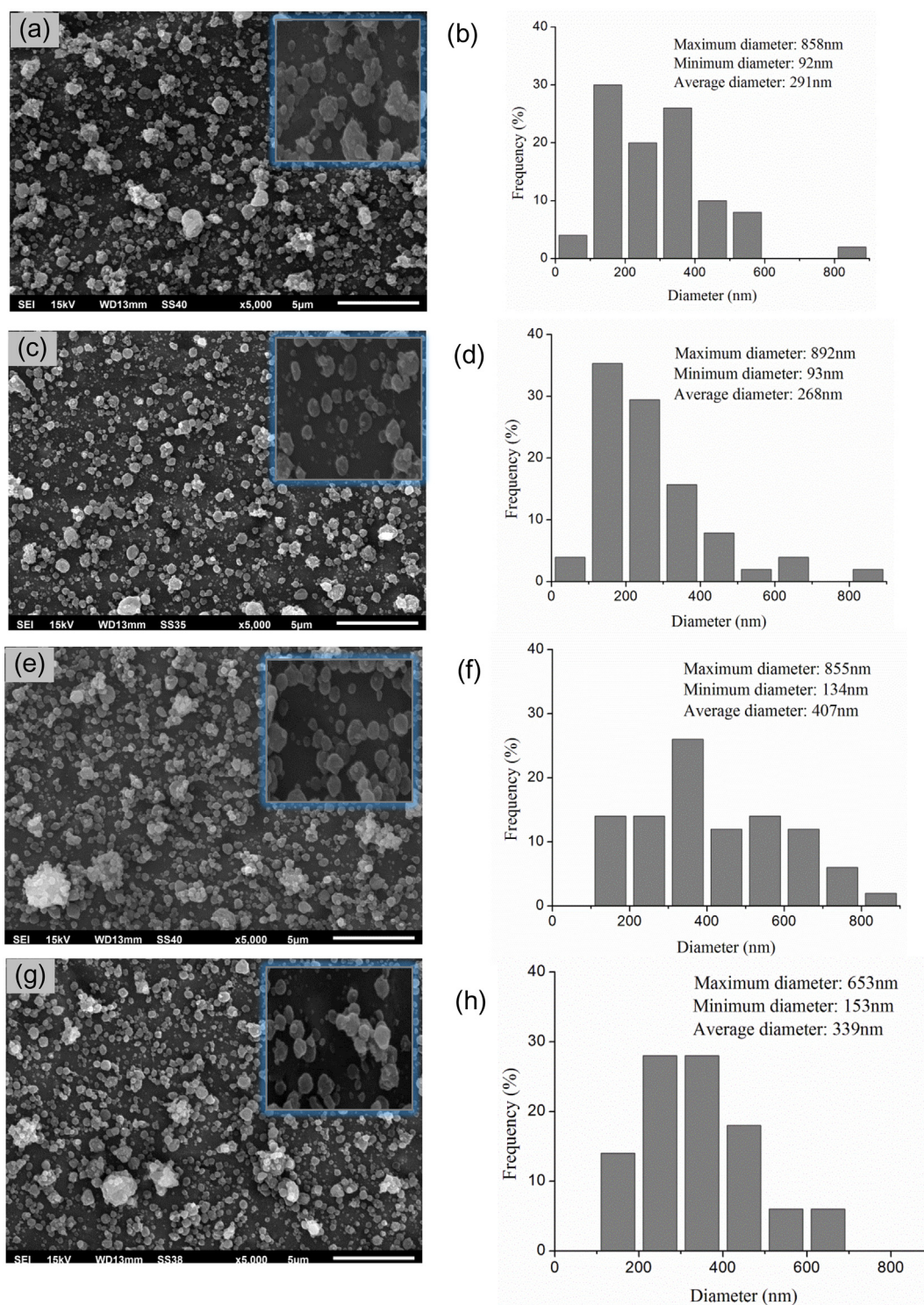


Fig. 3. Morphology of the capsules and their size distribution: pure zein (a, b), zein with 0.5% folic acid (c, d), zein with 1.0% folic acid (e, f) and zein with 1.5% folic acid (g, h). Image with amplitude of 30,000 × in the upper right corner of the SEM.

irradiation and in the absence of irradiation over a period of 60 days. They observed that when folic acid was encapsulated with the protein, folic acid concentration was reduced by 41% when exposed to irradiation; whereas in the absence of irradiation, the degradation was only 9%. In addition, folic acid encapsulated with resistant starch showed 88% degradation in the presence of irradiation, and in the dark this degradation was reduced to 37%.

3.6. Folic acid degradation products

Based on the results of folic acid resistance, it was observed that exposure of unencapsulated folic acid to either temperature or irradiation promotes vitamin degradation. Therefore, the degradation compounds formed in these processes were investigated. A temperature of 180 °C and exposure time to irradiation of 24 h were the parameters chosen for product identification, since these treatments are the most drastic and show the greatest degradation of folic acid.

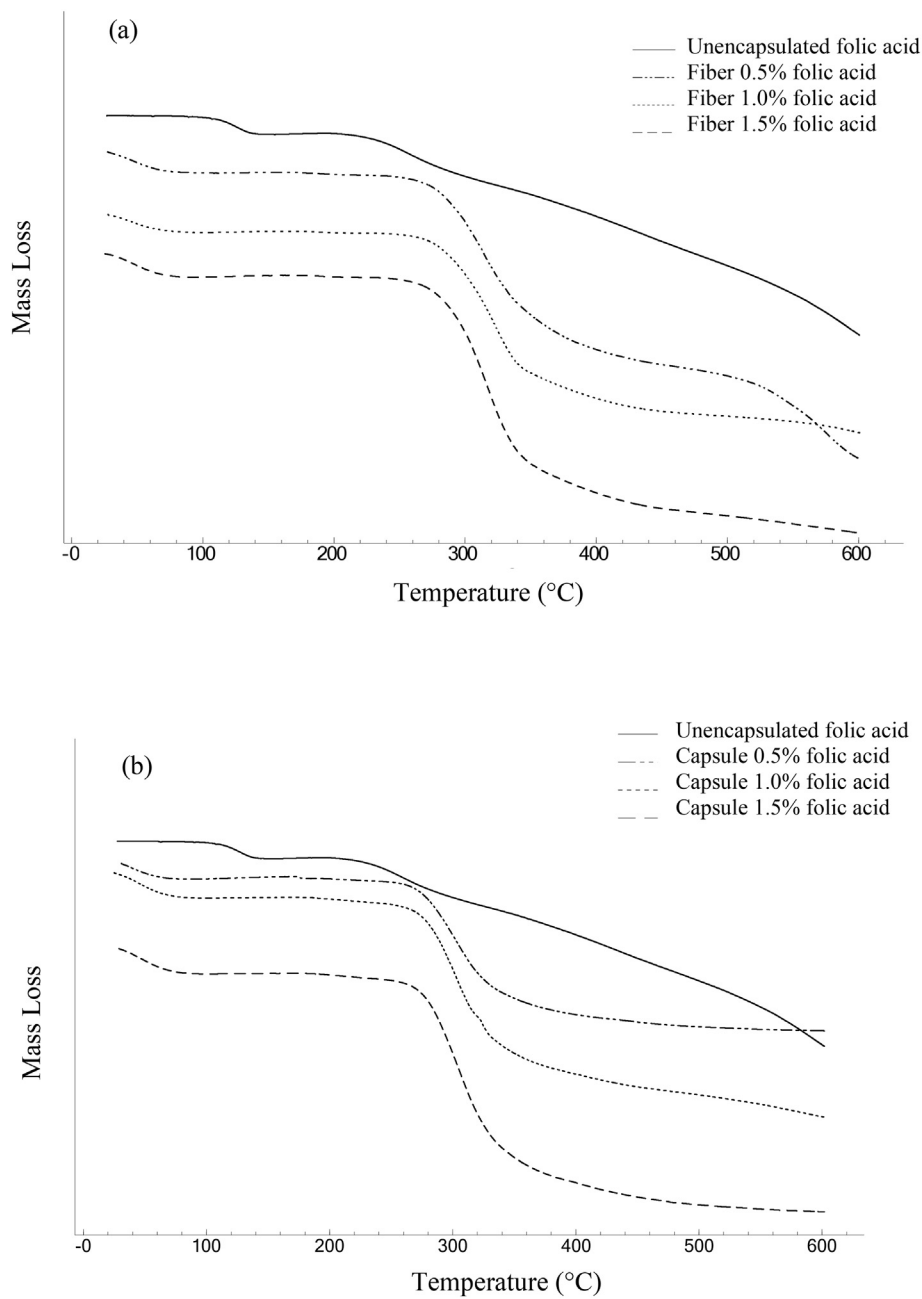


Fig. 4. TGA curves: (a) pure folic acid and zein fibers with 0.5, 1.0, and 1.5% folic acid, (b) pure folic acid and zein capsules with 0.5, 1.0, and 1.5% folic acid.

Table 2

Encapsulation efficiency and thermal resistance of encapsulated folic acid in the form of zein fibers or capsules.

Form of encapsulated	Folic acid (%)	Encapsulation efficiency (%) [*]	No exposure	Thermal treatment (°C)		
				100	140	180
				Folic acid (µg/mL) ^{**}		
Unencapsulated folic acid	–	–	17.17 ± 1.35 ^a	17.62 ± 1.98 ^{ab}	15.60 ± 0.14 ^b	5.44 ± 0.08 ^c
Fibers	0.5	92.9 ± 0.1 ^a	2.91 ± 0.15 ^a	2.71 ± 0.25 ^a	2.85 ± 0.03 ^a	2.67 ± 0.16 ^a
	1.0	91.7 ± 0.1 ^a	6.38 ± 0.13 ^a	6.53 ± 0.44 ^a	6.28 ± 0.36 ^a	6.00 ± 0.12 ^a
	1.5	82.1 ± 0.1 ^b	9.73 ± 1.20 ^a	9.10 ± 0.94 ^{ab}	7.95 ± 0.37 ^b	8.88 ± 0.07 ^{ab}
Capsules	0.5	98.6 ± 2.3 ^a	2.74 ± 0.15 ^a	2.84 ± 0.22 ^a	2.83 ± 0.08 ^a	2.84 ± 0.05 ^a
	1.0	92.9 ± 0.3 ^a	6.22 ± 0.03 ^a	5.63 ± 0.24 ^b	5.63 ± 0.21 ^b	5.60 ± 0.01 ^b
	1.5	73.7 ± 1.7 ^b	8.84 ± 0.55 ^a	7.26 ± 0.19 ^b	7.49 ± 0.06 ^b	7.69 ± 0.27 ^b

^{*} Encapsulation efficiency: Values with different letters in the same column for fibers or capsules are significantly different by Tukey's test ($p < .05$).

^{**} Folic acid content: Values with different letters in the same row are significantly different by Tukey's test ($p < .05$).

Table 3
Encapsulation efficiency and UVA irradiation resistance of encapsulated folic acid in the form of zein fibers or capsules.

Form of encapsulation	Folic acid (%)	Encapsulation efficiency (%) ^a	No exposure	UVA irradiation time (h)		
				1	12	24
Folic acid ($\mu\text{g/mL}$) ^{**}						
Unencapsulated folic acid		–	17.17 \pm 1.35 ^a	12.58 \pm 0.62 ^b	12.05 \pm 1.44 ^b	14.07 \pm 0.44 ^b
Fibers	0.5	92.9 \pm 0.1 ^a	2.91 \pm 0.15 ^a	2.59 \pm 0.33 ^a	2.93 \pm 0.29 ^{ab}	2.27 \pm 0.13 ^b
	1.0	91.7 \pm 0.1 ^a	6.38 \pm 0.13 ^a	5.05 \pm 0.32 ^{ab}	5.05 \pm 0.88 ^b	4.85 \pm 0.58 ^b
	1.5	82.1 \pm 0.1 ^b	9.73 \pm 1.20 ^a	8.02 \pm 1.00 ^{ab}	7.92 \pm 0.48 ^b	8.88 \pm 0.05 ^{ab}
Capsules	0.5	98.6 \pm 2.3 ^a	2.74 \pm 0.15 ^a	2.50 \pm 0.16 ^a	2.68 \pm 0.13 ^a	2.50 \pm 0.30 ^a
	1.0	92.9 \pm 0.3 ^a	6.22 \pm 0.03 ^a	5.04 \pm 0.53 ^{ab}	4.67 \pm 0.30 ^b	5.56 \pm 1.1 ^{ab}
	1.5	73.7 \pm 1.7 ^b	8.84 \pm 0.55 ^a	7.75 \pm 0.51 ^a	7.93 \pm 1.30 ^a	7.88 \pm 1.60 ^a

^a Encapsulation efficiency: Values with different letters in the same column for fibers or capsules are significantly different by Tukey's test ($p < .05$).

^{**} Folic acid content: Values with different letters in the same row are significantly different by Tukey's test ($p < .05$).

Folic acid was identified by the presence of the $[\text{M} + \text{H}]^+ m/z$ 442.1469. The content of the folic acid submitted to the heat treatment was lower when compared to the folic acid control (without thermal treatment). In addition, the degradation products including 6-carboxypterin (PCA), p-aminobenzoyl-L-glutamic acid (pABGA), p-aminobenzoic acid (PABA), and pteric acid (PA) were identified by the presence of the $[\text{M} + \text{H}]^+ m/z$ 208.0465, 267.0975, 138.0549, and 313.1043, respectively. Vora et al. (2002) studied the thermostability of folic acid and suggested that initially the glutamate fraction of folic acid breaks, leaving the amide as the major component. Then the pterin and PABA decompose in an overlapping mechanism. These authors also reported that at 195 °C it is not possible to discern the groups present, indicating a complete decomposition of the vitamin at this temperature. According to Ball (2006), folic acid has no defined melting point, but at 250 °C an important degree of carbonization is already observed.

The degradation compounds pABGA ($[\text{M} + \text{H}]^+ m/z$ 267.0975) and PA ($[\text{M} + \text{H}]^+ m/z$ 313.1043) were identified after exposition of folic acid to UVA irradiation for 24 h. The pABGA is formed by the photochemical cleavage of the C9-N10 bond, while the PA is the product of amide linkage of folic acid between the glutamate and PABA. Akhtar, Khan, and Ahmad (1999, 2003) studied the effects of different pH on photodegradation of folic acid by UV and found PABA, PCA, pABGA, and PA as its major degradation products.

4. Conclusion

Polymer solutions of zein containing encapsulated folic acid were able to form continuous and uniform ultrafine fibers and nanocapsules by electrospinning and electrospaying processes. Through thermogravimetric analysis, it was possible to observe an increase in the thermal resistance of folic acid when it was incorporated in the fibers or capsules. Zein and fiber capsules showed high encapsulation efficiency for folic acid (> 80%). Unencapsulated folic acid showed intense degradation when exposed to 180 °C (70% loss); however, while encapsulated in fiber or capsules the vitamin was stable. The photodegradation of unencapsulated folic acid was approximately 26% after 1 h of UVA irradiation; however, when folic acid was incorporated into zein fibers, radiation resistance was generally observed. Zein fibers and capsules with folic acid present promising characteristics for application in foods that require thermal processing and exposure to irradiation.

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Conflict of interest

The authors declare that there is no conflict of interest.

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