**Photoprotection of Folic Acid upon Encapsulation in Food-Grade Amaranth
(*Amaranthus hypochondriacus* L.) Protein Isolate - Pullulan Electrospun Fibers**

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Abbreviations Used

API, amaranth protein isolate; UV, ultraviolet; FTIR, Fourier-transform infrared; TG, thermogravimetric;
DTG, derivative thermogravimetric; PGA, *p*-aminobenzoyl glutamic acid; FPT, 6-formylpterin.

Abstract

In this work, the ability of amaranth protein isolate (API):pullulan structures obtained through electrospinning for the photoprotection of bioactive compounds was studied. The model bioactive compound encapsulated was folic acid, due to its great sensitivity to UV light exposure. Addition of 100 mg of folic acid per g of biopolymer to the biopolymeric solution used for electrospinning resulted in increased apparent viscosity and, thus, in thicker electrospun fibers. Very high encapsulation efficiency was obtained (>95%) using this encapsulation technology and no specific chemical interactions were established between the vitamin and the matrix materials as inferred from FTIR analysis. Encapsulation within the API:pullulan structures increased thermal stability of folic acid, which may be useful for food processing applications. Furthermore, no degradation of the encapsulated compound was observed after 2 hours of UV exposure, while the characteristic UV-Vis spectrum from the photodegradation compounds of folic acid was observed after UV irradiation of the unprotected vitamin.

Keywords

Electrospinning, vitamin B9, microencapsulation, UV radiation, biopolymers

Chemical compounds

Folic acid (PubChem CID: 6037)

1. Introduction

Folic acid or pteroylmonoglutamate (PteGlu) is a stable synthetic analog of the natural folates family. The interest in this water-soluble B vitamin is derived from its beneficial in preventing a range of disorders, not only in its native form, but also as a dietary or pharmacological supplement. This vitamin is of paramount importance in biochemical processes related with DNA synthesis and repair (Lucock, 2000). In fact, folate deficiency can derive in a series of health disorders like neural tube defects, several cancers (cervical, bronchial, colon and breast), Alzheimers disease, affective disorders, Down's syndrome, and pregnancy-related complications (Off et al., 2005). Moreover, the scientific evidences that link the preventative role associated to a greater folic acid intake, have seconded the recommendations prescribed by the Public Health Service from USA in 1992 and supported by the FAO/WHO experts consultation in 1998, that all women in reproductive age should consume 0.4 mg of synthetic folic acid apart from a natural folate-rich diet (FAO/WHO, 2002). However, reaching the recommended intake level of natural folates through the diet is difficult given their low bioavailability, while supplementing with synthetic folic acid the whole population in risk constitutes a great logistic challenge, even in the developed countries. Therefore, food fortification with folic acid can be a good strategy to increase the basal folate intake levels. From an industrial point of view, folic acid fortification has been devised as an adequate intervention in, for instance, flours, as it is technologically feasible, economically viable and it does not alter organoleptic properties at the concentrations added (Sanabria & Tarqui, 2007). Unfortunately, the great instability of folic acid when exposed to light and other ambient factors represents a problem for industrial handling and, thus, strategies to diminish photodegradation, allowing a better availability of this bioactive compound are sought. Micro- and nanoencapsulation are plausible options that have

been recently explored for food fortification and folic acid and derivatives have been microencapsulated using starch, alginate and/or pectins through spray-drying (Liu, Green, Wong, & Kitts, 2012; Madziva, Kailasapathy, & Phillips, 2006; Shrestha, Arcot, & Yuliani, 2012). Microencapsulation resulted in improved stability of the bioactive within various food matrices and during food processing. Smaller capsule morphologies have been also developed through other encapsulation techniques, like ionic gelation (de Britto, de Moura, Aouada, Mattoso, & Assis, 2012), or electrospinning (Bakhshi, Nangrejo, Stride, & Edirisinghe, 2013), but in these works, only the optimization of the encapsulation process and characterization of the capsules was reported, while no information about the stability of folic acid was provided. In the last years, electrospinning has been broadly explored as a straightforward and versatile method for encapsulation, with a number of advantages when compared to traditional encapsulation techniques such as spray drying, coacervation or ionic gelation. The most interesting advantage of electrospinning for encapsulation applications is that it does not require severe conditions, both in terms of temperature and solvents used, giving rise to smaller capsule sizes and, in general, showing high encapsulation efficiencies (Bhushani & Anandharamakrishnan, 2014; Zussman, 2011). Recently, we reported about the development of novel amaranth (*Amaranthus hypochondriacus L.*) protein-based electrospun fibers using a food contact solvent (Aceituno-Medina, Lopez-Rubio, Mendoza, & Lagaron, 2013a; Aceituno-Medina, Mendoza, Lagaron, & Lopez-Rubio, 2013b). Amaranth is a traditional under-utilized Mexican crop with highly nutritious grains and leafs. The aim of this work was to investigate the potential of these novel amaranth-based structures for the encapsulation and photoprotection of folic acid for food fortification. To the best of our knowledge, this is the first time that an amaranth protein-based matrix is used for the encapsulation and protection of bioactives. The

morphology of the developed electrospun fibers together with the encapsulation efficiency and bioactive stability were studied. Moreover, the photostability of this vitamin when exposed to UV irradiation was also investigated and compared to that of the free compound.

2. Materials and methods

2.1 Materials

Formic acid of 95% purity, non-ionic surfactant, polyoxyethylene sorbitan monooleate (Tween 80), folic acid (>97% purity), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulfate and pullulan ($M_w \sim 100000$) were supplied by Sigma-Aldrich. All products were used as received, without further purification. The commercial amaranth protein concentrate (*Amaranthus hypochondriacus* L. Revancha variety) was supplied by Nutrisol (Hidalgo, Mexico). The Amaranth Protein Isolate (API) was prepared based on the methodology previously reported by Martínez and Añón (1996) with some modifications. The protein isolate prepared under these conditions consisted in a mixture of different proteins with molecular weights ranging from 10-83 kDa (Aceituno-Medina et al., 2013a). Briefly, the commercial amaranth protein concentrate (APC) was defatted with hexane for 12 h (100 g/l suspension). Then, the amaranth protein concentrate was suspended in water and its pH was adjusted to 9 with a 2 mol/l NaOH solution. The suspension was stirred for 30 min at room temperature and, then, centrifuged 20 min at 9000 x g. Then, the supernatant was adjusted to pH 5 with 2 mol/l HCl and centrifuged at 9000 x g for 20 min at 4°C. The pellet was resuspended in water, neutralized with 0.1 mol/l NaOH and freeze-dried. The protein content was determined by the Kjeldahl technique (AOAC, 1996) using a conversion factor of 5.85.

2.2 Preparation of folic acid-containing solutions for electrospinning

In order to develop the electrospun fibers for encapsulation of folic acid, a blend of 80:20 API:pullulan with the surfactant Tween 80 (~200 mg/g of API) was prepared using 95% formic acid as the solvent. The total polymer content in solution was 200 g/l. The amount of folic acid incorporated into the solution was 100 mg/g of biopolymer blend. The solutions were gently stirred until homogeneous dispersions were obtained.

2.3 Characterization of the polymer solutions

The apparent viscosity (η_a) of the polymeric solutions at 100 s^{-1} was determined using a rotational viscosity meter Visco Basic Plus L from Fungilab S.A. (San Feliu de Llobregat, Spain) using a Low Viscosity Adapter (LCP). The measurements were made in triplicate at 25°C.

2.4 Development of encapsulation structures through electrospinning

The methodology to obtain API/pullulan fibers through electrospinning has been described elsewhere (Aceituno-Medina et al., 2013b). All of the electrospinning experiments were carried out at room temperature in air. The electrospinning environmental conditions were maintained stable at 24°C and 60% RH by having the equipment enclosed in a specific chamber with temperature and humidity control. In this work, the specific conditions of the electrospinning process for obtaining the fibers loaded with folic acid were: a tip-to-collector distance of 10 cm, a flow rate of the solution of 0.4 ml/h and the voltage was kept at 22 kV.

2.5 Optical and Scanning Electron Microscopy (SEM)

The presence and distribution of folic acid within the electrospun API/pullulan fibers were observed using a digital microscopy system (Nikon Eclipse 90i) fitted with a 12 V, 100 W halogen lamp and equipped with a digital imaging head which integrates an epifluorescence illuminator. A digital camera head (Nikon DS-5Mc) with a 5 megapixel CCD cooled with a Peltier mechanism was attached to the microscope. Nis Elements software (Nikon Instruments Inc., Melville, USA) was used for image capturing and the Adobe Photoshop CS3 extended software was used for image processing and analysis. The morphology of the folic acid-containing electrospun fibers was examined using SEM (Hitachi S-4100) after sputtering the samples with a gold–palladium mixture under vacuum. All SEM experiments were carried out at an accelerating voltage of 10 kV. Fiber diameters of the electrospun fibers were measured by means of the Adobe Photoshop 7.0 software from the SEM micrographs in their original magnification.

2.6 Attenuated total reflectance infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectra of the electrospun fibers were collected in a controlled chamber at 24°C and 40% RH coupling the ATR accessory GoldenGate of Specac Ltd. (Orpington, UK) to a Bruker (Rheinstetten, Germany) FTIR Tensor 37 equipment. All the spectra were collected by averaging 20 scans at 4 cm⁻¹ resolution. Analysis of the spectral data was performed using Grams/AI 7.02 (Galactic Industries, Salem, NH, USA) software.

2.7 Encapsulation efficiency

To assess the bioactive encapsulation yield, approximately 3 mg of loaded fibers were placed in a Falcon tube with 5 ml of PBS buffer pH 7 and stirred during 10 min at ~12 x g to remove the bioactive from the surface of the encapsulation structures. Then, the tubes were centrifuged at 10000 x g during 10 min at 20°C. The supernatant was taken

and stored for subsequent analysis and the precipitate was repeatedly re-suspended in 5 ml of PBS and centrifuged as explained above until no signal from the bioactive was obtained. The amount of folic acid was monitored through UV-Vis spectrophotometry using a SP-2000UV spectrophotometer, through interpolation of the absorbance maximum of folic acid at 348 nm within the calibration curve previously obtained. The study was performed in triplicate.

2.8 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TG) curves were recorded with a TGA-DTA Setaram Setsys equipment. The samples (ca. 10 mg) were heated from 50 to 800°C with a heating rate of 5°C/min under argon atmosphere.

2.9 Photostability of folic acid by UV-Vis irradiation

In order to determine the photostability of encapsulated folic acid when exposed to UV radiation, 2 mg of fibers containing folic acid (~140 µg) and the same amount of free folic acid (i.e. ~140 µg) were placed in 5 ml of PBS buffer pH 7 and these solutions were placed under an Osram Ultra-Vitalux (300 W) lamp during 120 min to accelerate folic acid oxidation. A control solution with the same amount of loaded fibers was kept in dark conditions for comparison purposes. After irradiation, folic acid was extracted from the fibers through centrifugation at 10000 x g during 10 minutes at 20°C and oxidation was quantified through UV-Vis spectrophotometry at the absorbance maximum of folic acid (~348 nm). The study was performed in triplicate.

3. RESULTS AND DISCUSSION

3.1 Morphology of the encapsulation structures and distribution of the bioactive within the electrospun fibers

Using the electrospinning technique, it was possible to develop homogeneous ultrathin fibers from blends of an amaranth protein isolate (API) and the spinnable carbohydrate pullulan containing up to 0.8 g API per gram of blend upon addition of 200 mg of the non-ionic surfactant Tween 80 per gram of biopolymer blend (Aceituno-Medina et al., 2013b). Using this high protein content composition, encapsulation structures were developed which contained 100 mg of folic acid per gram of biopolymer and the effect of bioactive addition on the morphology of the fibers was studied. Figure 1 shows the SEM and optical microscopy images of the neat and loaded fibers. Upon addition of folic acid, the apparent viscosity of the solution increased, which resulted in thicker average diameters of the fibers as expected (Bhardwaj & Kundu, 2010). Nevertheless, smooth and rather homogeneous fibers in terms of size distribution were obtained, indicating that incorporation of the bioactive did not negatively affect the electrospinning process.

INSERT FIGURE 1 ABOUT HERE

Figures 1C and 1D show the optical microscopy images of the loaded fibers which confirm the rather homogeneous size distribution of the electrospun fiber diameters. The chemical structure of folic acid consists in pteric acid, 4-[(pteridin-6-ylmethyl) amino] benzoic acid conjugated with glutamate, with the pteridin moiety being responsible for its fluorescence at 455 nm (Liang & Subirade, 2010). Therefore, distribution of the vitamin within the electrospun fibers was studied using fluorescence

microscopy. From the fluorescence microscopy image (Figure 1D), a good distribution of folic acid was observed along the fibers, although some agglomerated areas could also be discerned.

3.2 Encapsulation efficiency

To evaluate the encapsulation efficiency of the folic acid within the electrospun API:pullulan fibers, a calibration curve of folic acid in PBS pH7 was performed and the following calibration curve was obtained:

$$y = 0.0155x + 0.0049 \quad (R^2 = 0.995) \quad (\text{Equation 1})$$

Where x was the amount of folic acid ($\mu\text{g/ml}$) and y the absorbance at 348 nm.

The amount of folic acid effectively encapsulated was then monitored through UV-Vis spectrophotometry through interpolation of the absorbance maximum at 348 nm within the calibration curve previously obtained. The theoretical concentration of folic acid within the fibers was $79.3 \mu\text{g/mg}$ of fiber, while the real concentration obtained was $75.8 (\pm 0.2) \mu\text{g/mg}$, thus resulting in an encapsulation efficiency of $95.6\% (\pm 0.2)$. It is interesting to note that greater encapsulation efficiency was obtained in this work using electrospinning than in previous works using spray drying, where encapsulation efficiencies ranging from ~25 to 89% were observed (Liu et al., 2012; Shrestha et al., 2012). Moreover, regarding the loading capacity of the API:pullulan fibers, considerably greater amounts of folic acid ($\sim 76 \mu\text{g/mg}$) were present compared with the concentration of vitamin encapsulated in alginate:pectin matrices, in which $374 \mu\text{g/g}$ (Shrestha et al., 2012) and $0.316 \mu\text{g/g}$ (Madziva et al., 2006) were effectively incorporated.

3.3 Spectroscopic analyses

Infrared spectroscopy was used as a tool to confirm the correct encapsulation of the folic acid within the electrospun fibers, as well as to study potential interactions between the bioactive and the API:pullulan matrix material. Figure 2 shows the ATR-FTIR spectra of pure folic acid, neat fibers and loaded fibers, in which only small contributions from the bending mode vibration of the N-H group of the vitamin (at $\sim 1604\text{ cm}^{-1}$) and from the band at $\sim 1400\text{ cm}^{-1}$ were discerned (cf. the magnified spectra and arrows in Figure 2), due to the overlapping of the characteristic spectral bands of folic acid with those from the API protein and pullulan matrix.

INSERT FIGURE 2 ABOUT HERE

In general, no significant changes in the secondary structure of the protein, neither spectral band displacements were observed upon folic acid encapsulation, although the broadening of the band associated to -OH stretching (from $3000\text{-}3600\text{ cm}^{-1}$) could suggest the formation of hydrogen bonds between the fiber components (Chen, Xiumei, & Fengling, 2007).

3.4 Thermal stability of folic acid

Thermogravimetric analysis was carried out to ascertain if the encapsulation process of folic acid within the electrospun API:pullulan fibers, affected the thermal stability of the bioactive. Figure 3 shows the thermogravimetric (TG) and derivative thermogravimetric (DTG) curves of folic acid, neat API:pullulan electrospun fibers and folic acid-loaded fibers. Table 1 compiles the maximum of the main degradation bands obtained from the weight loss first derivative curves.

INSERT FIGURE 3 ABOUT HERE

The derivative thermogravimetric curve from pure folic acid showed four degradation stages. The first one, around 100°C, is due to the loss of adsorbed water and it was seen to disappear upon encapsulation. The other three degradation events appeared highly overlapped and, specifically, the third and fourth maximum degradation temperatures were close to those from the API:pullulan and surfactant Tween 80, respectively (Aceituno-Medina et al., 2013b). It has been described that, during degradation of folic acid, first, the glutamic acid component breaks away from the folic acid structure leaving the amide as a major constituent. Then the amide, pterin, degrades along with the para-aminobenzoic acid in an overlapping mechanism (Vora, Riga, & Alexander, 2002).

INSERT TABLE 1 ABOUT HERE

Encapsulation of folic acid within the API:pullulan electrospun fibers, effectively increased the thermal stability of folic acid, as the second degradation peak from the pure compound at ~244°C shifted to higher temperatures and was only discerned as a shoulder just before the main degradation peak from the API: pullulan at ~215°C (cf. Figure 3B). As observed in Figure 3 and Table 1, the degradation temperatures of the encapsulating matrices and surfactant were not significantly modified in the loaded structures. Therefore, it seems that incorporation of folic acid within the API:pullulan fibers through electrospinning contributed to a thermal stabilization of the compound.

3.5 Photostability of encapsulated folic acid

One of the main objectives of this work was to check the photoprotection ability of the API:pullulan hybrid electrospun fibers. Folic acid is characterized by its great sensitivity to UV light, which causes the excision of the bond between the carbon placed in position 9 and the nitrogen in position 10 (Akhtar, Khan, & Ahmad, 2003; Off et al., 2005), i.e. cleaving the pteridine from the rest of the molecule. Therefore, both the pure compound and the encapsulated folic acid were exposed to UV radiation and the UV-Vis spectra were compared with that of the non-irradiated compound. For UV-Vis analysis, folic acid was extracted from the electrospun structures using PBS pH7. Figure 4 shows the UV-Vis spectra of the non-encapsulated and encapsulated folic acid before and after 120 min exposure to a UV lamp.

INSERT FIGURE 4 ABOUT HERE

Folic acid absorbs light in the UV region, having absorption peaks at ~280 and ~360 nm. From Figure 4 it can be observed that non-protected folic acid was degraded upon UV exposure, which provoked a change in its absorption spectrum and the maximums of absorption were shifted towards shorter wavelengths. It is worth mentioning that these changes were observed after only 60 min of UV exposure in PBS solution (results not shown). Thomas, Suarez, Cabrerizo, Martino, & Capparelli (2000) proposed that *p*-aminobenzoyl glutamic acid (PGA) and 6-formylpterin (FPT) were the photoproducts of folic acid. PGA has an absorption peak at 275 nm, while FPT absorbs in the entire UV region with small peaks at 278, 310 and 365 nm (Off et al., 2005). The spectrum from UV irradiated non-encapsulated folic acid seems to be a combination of the photodegradation products previously described. In contrast, the folic acid extracted from the encapsulation structures after UV exposure, maintains its absorption spectrum,

indicating that the API:pullulan matrix was effective in protecting the photosensitive molecule from UV radiation.

Conclusions

Folic acid has been encapsulated through electrospinning in amaranth protein isolate (API):pullulan ultrathin fibers with very high encapsulation efficiency (>95%). Even though no specific chemical interactions were established between the vitamin and the matrix materials as inferred from FTIR analysis, an increase in the thermal stability of folic acid was observed which may be useful for food processing applications. Furthermore, no degradation of the encapsulated compound was observed after 2 hours of UV exposure, while the characteristic UV-Vis spectrum from the photodegradation compounds of folic acid was observed after UV irradiation of the unprotected vitamin. Therefore, the API:pullulan structures have demonstrated a great potential for the encapsulation and protection of photosensitive bioactives for food-related applications.

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References

- Aceituno-Medina, M., Lopez-Rubio, A., Mendoza, S., & Lagaron, J. M. (2013a). Development of novel ultrathin structures based in amaranth (*Amaranthus hypochondriacus*) protein isolate through electrospinning. *Food Hydrocolloids*, 31, 289-298.
- Aceituno-Medina, M., Mendoza, S., Lagaron, J. M., & Lopez-Rubio, A. (2013b). Development and characterization of food-grade electrospun fibers from amaranth protein and pullulan blends. *Food Research International*, 54, 667-674.
- Akhtar, M. J., Khan, M. A., & Ahmad, I. (2003). Identification of photoproducts of folic acid and its degradation pathways in aqueous solution. *Journal of Pharmaceutical and Biomedical Analysis*, 31, 579-588.
- Association of Official Analytical Chemists (1996). Official methods of analysis (16th ed.), Arlington, VA: AOAC.
- Bakhshi, P. K., Nangrejo, M. R., Stride, E., & Edirisinghe, M. (2013). Application of electrohydrodynamic technology for folic acid encapsulation. *Food and Bioprocess Technology*, 6, 1837-1846.
- Bhardwaj, N., & Kundu, S. C. (2010). Electrospinning: a fascinating fiber fabrication technique. *Biotechnology Advances*, 28, 325-347.
- Bhushani, J. A., & Anandharamakrishnan, C. (2014). Electrospinning and electrospraying techniques: potential food based applications. *Trends in Food Science and Technology*, 38, 21-33.
- Chen, Z., Xiumei, M., & Fengling, Q. (2007). Electrospinning of collagen–chitosan complex. *Materials Letters*, 61, 3490–3494.

351 de Britto, D., de Moura, M. R., Aouada, F. A., Mattoso, L. H. C., & Assis, O. B. G.
 352 (2012). N,N,N-trimethyl chitosan nanoparticles as a vitamin carrier system. *Food*
 353 *Hydrocolloids*, 27, 487-493.
 354 FAO/WHO (2002). Folate and Folic Acid. In: *Human Vitamin and Mineral*
 355 *Requirements - Report of a joint FAO/WHO expert consultation* (pp. 53-62). Bangkok,
 356 Thailand, 2002. Online document. [ftp://ftp.fao.org/docrep/fao/004/y2809e/](ftp://ftp.fao.org/docrep/fao/004/y2809e/y2809e00.pdf)
 357 [y2809e00.pdf](ftp://ftp.fao.org/docrep/fao/004/y2809e/y2809e00.pdf). Accessed 5 August 2014.
 358 Liang, L., & Subirade, M. (2010). β -Lactoglobulin/Folic Acid Complexes: Formation,
 359 Characterization, and Biological Implication. *Journal of Physical Chemistry B*, 114,
 360 6707–6712.
 361 Liu, Y., Green, T. J., Wong, P., & Kitts, D. D. (2012). Microencapsulation of L-5-
 362 methyltetrahydrofolic acid with ascorbate improves stability in baked bread products.
 363 *Journal of Agricultural and Food Chemistry*, 61, 247-254.
 364 Lucock, M. (2000). Folic acid: nutritional biochemistry, molecular biology, and role in
 365 disease processes. *Molecular Genetics and Metabolism*, 71, 121-138.
 366 Madziva, H., Kailasapathy, K., & Phillips, M. (2006). Evaluation of alginate-pectin
 367 capsules in Cheddar cheese as a food carrier for the delivery of folic acid. *LWT-Food*
 368 *Science and Technology*, 39, 146-151.
 369 Martínez, E. N., & Añón, M. C. (1996). Composition and structural characterization of
 370 amaranth protein isolates. An electrophoretic and calorimetric study. *Journal of*
 371 *Agricultural and Food Chemistry*, 44, 2523–2530.
 372 Off, M. K, Steindal, A. E., Porojnicu, A. C., Juzeniene, A., Vorobey, A., Johnsson, A.
 373 & Moan, J. (2005). Ultraviolet photodegradation of folic acid. *Journal of*
 374 *Photochemistry and Photobiology B-Biology*, 80, 47-55.

375 Sanabria, H., & Tarqui, C. (2007). Fundamentos para la fortificación de la harina de
 376 trigo con micronutrientes en el Perú. *Anales de la Facultad de Medicina*, 68(2), 185-
 377 192.

378 Shrestha, A. K., Arcot, J., & Yuliani, S. (2012). Susceptibility of 5-
 379 methyltetrahydrofolic acid to heat and microencapsulation to enhance its stability
 380 during extrusion processing. *Food Chemistry*, 130, 291-298.

381 Thomas, A. H., Suarez, G., Cabrerizo, F. M., Martino, R., & Capparelli, A. L. (2000).
 382 Study of the photolysis of folic acid and 6-formylpterin in acid aqueous solutions.
 383 *Journal of Photochemistry and Photobiology A - Chemistry*, 135, 147–154.

384 Vora, A., Riga, A., & Alexander, K. (2002). Processes to identify the degradation
 385 mechanism of a solid which appears to undergo a complex reaction: folic acid.
 386 *Instrumentation Science & Technology*, 30, 193-203.

387 Zussman, E. (2011). Encapsulation of cells within electrospun fibers. *Polymers for*
 388 *Advanced Technologies*, 22, 366-371.

Table 1 Maximums of the weight loss first derivate (TD) obtained from the thermogravimetric analysis for the folic acid and for the neat and loaded amaranth protein isolate (API):pullulan electrospun fibers

Sample	T _{max1} (°C)	T _{max2} (°C)	T _{max3} (°C)	T _{max4} (°C)	Reference
Folic acid	95.3	244.1	304.9	399.6	
API:pullulan	310.9	407.3			Aceituno-Medina et al. (2013)
API:pullulan (folic acid)	314.6	407.5			

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Figure Captions

Fig. 1 Scanning electron microscopy (SEM) (A,B) and optical microscopy images under polarized light (C) and using a fluorescence source (D) of: 80:20 amaranth protein isolate:pullulan fibers without bioactive (A) and containing 100 mg of folic acid per gram of biopolymer blend (B-D). Scale markers correspond to 2 μm (A, B) and 20 μm (C, D). SEM micrographs also include the apparent viscosity (η_{app}) of the electrospinning solutions and average diameter of the fibers obtained

Fig 2 Attenuated total reflectance infrared (ATR-FTIR) spectra of: (A) Folic acid; (B) amaranth protein isolate:pullulan fibers; (C) amaranth protein isolate:pullulan fibers with 100 mg of folic acid per gram of biopolymer blend. Arrows indicate the main spectral differences observed in the folic acid-loaded electrospun fibers. Spectra have been offset and an inset showing the amplified FTIR region from 1700 to 1300 cm^{-1} has been also included for clarity

Fig 3 Thermogravimetric (A) and derivative thermogravimetric curves (B) of folic acid (dashed line), neat amaranth protein isolate:pullulan fibers (continuous line) and folic acid-loaded fibers (dash-dot line)

Fig 4 UV-Vis spectra of pure folic acid before (continuous line) and after 120 min exposure to UV light (dotted line) and folic acid encapsulated within amaranth protein isolate:pullulan electrospun fibers before (dashed line) and after 120 min exposure to UV light (dash-dot line)

FIGURE 1.

FIGURE 2

FIGURE 2 INSET

FIGURE 3A.

FIGURE 3B.

FIGURE 4.

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397 **HIGHLIGHTS**

- 398 • Folic acid was encapsulated in amaranth protein isolate:pullulan matrices
- 399 • Electrospinning was the encapsulation technique used
- 400 • Very high encapsulation efficiency was obtained (>95%)
- 401 • Encapsulation led to enhanced folic acid thermal stability
- 402 • The biopolymer matrices also protected folic acid from UV photodegradation
- 403