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# Photoprotection of folic acid upon encapsulation in food-grade amaranth (*Amaranthus hypochondriacus* L.) protein isolate – Pullulan electrospun fibers

Marysol Aceituno-Medina [a], Sandra Mendoza [a], José María Lagaron [b], Amparo López-Rubio [b, *]

[a] Dept. de Investigación y Posgrado en Alimentos, Facultad de Química, Universidad Autónoma de Querétaro, 76010, Querétaro, Mexico
[b] Novel Materials and Nanotechnology Group, IATA-CSIC, Avda. Agustin Escardino 7, 46980, Paterna (Valencia), Spain

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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 h 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.

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## 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

*Abbreviations:* API, Amaranth protein isolate; UV, Ultraviolet; FTIR, Fourier-transform infrared; TG, Thermogravimetric; DTG, Derivative thermogravimetric; PGA, *p*-Aminobenzoyl glutamic acid; FPT, 6-Formylpterin.

* Corresponding author. Tel.: +34 963900022; fax: +34 963636301.
*E-mail address:* amparo.lopez@iata.csic.es (A. López-Rubio).

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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, 2013; Aceituno-Medina, Mendoza, Lagaron, & Lopez-Rubio, 2013). Amaranth is a traditional underutilized 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 ($M_w$ ~ 100,000) were supplied by Sigma–Aldrich. All products were used as received, without further purification. The commercial amaranth protein concentrate (*A. 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 to 83 kDa (Aceituno-Medina, Lopez-Rubio, et al., 2013). 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 × *g*. Then, the supernatant was adjusted to pH 5 with 2 mol/l HCl and centrifuged at 9000 × *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 homogenous dispersions were obtained.

### 2.3. Characterization of the polymer solutions

The apparent viscosity ($\eta_a$) 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, Mendoza, et al., 2013). 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/pulllulan 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.

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