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Protection of folic acid through encapsulation in mesoporous silica particles included in fruit juices

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ABSTRACT

Folic acid (FA) is a synthetic vitamin commonly used for food fortification. However, its vulnerability to processing and storage implies loss of efficiency, which would induce over-fortification by processors to obtain a minimum dose upon consumption. Recent studies have indicated potential adverse effects of FA overdoses, and FA protection during processing and storage could lead to more accurate fortification. In addition, sustained vitamin release after consumption would help improve its metabolism. The objective of this work was to study controlled FA delivery and stability in fruit juices to reduce potential over-fortification risks by using gated mesoporous silica particles (MSPs). The obtained results indicated that FA encapsulation in MSPs significantly improved its stability and contributed to controlled release after consumption by modifying vitamin bioaccessibility. These results confirmed the suitability of MSPs as support for controlled release and protection of bioactive molecules in food matrices in different food production and storage stages.

1. Introduction

The generic term "folates" refers to a group of naturally-occurring B vitamins essential for the human body. An adequate folate status provides health benefits by preventing birth defects during pregnancy and cardiovascular diseases (Hoag, Ramachandruni, & Shangraw, 1997), Alzheimer's disease (Clarke et al., 1998) or colorectal cancer (Stover, 2004). The European Food Safety Authority (EFSA) recommends a daily intake of 200–400 μg folate/day for adults, and an additional intake of 400 μg for women of childbearing age (ESCO, 2009).

Folates are present in diverse food products, including liver, egg yolk, green vegetables, certain beans, citrus fruits, and cereal products (Ball, 2005). However, folate intake is strongly influenced by the degradation of its vitamers during food processing. Hence folic acid (FA), a synthetic form of the vitamin, is commonly used for folate supplementation and food fortification thanks to its reported enhanced bioaccessibility and stability. Food fortification should guarantee the FA concentration indicated on the label until the expiry date, which usually entails having to add large amounts of the vitamin (up to 50%) to compensate for the losses that occur during processing and/or storage (Frommherz et al., 2014). The folates degradation process depends on several factors, such as high temperature, light, low pH, oxygen and overall food composition (Fukuwatari, Fujita, & Shibata, 2009; Jastrebova, Axelsson, Strandler, & Jägerstad, 2013; Nguyen, Oey, Verlinde, van Loey, & Hendrickx, 2003). Some studies have reported a marked deviation of FA content from the labeled values of fortified foods (Frommherz et al., 2014; Lebiedzińska, Dbrowska, Szefer, & Marszałł, 2008), which can result in health risks. In particular, FA, which requires metabolic activation before it can function, appears unmetabolized in the bloodstream when the tolerable upper intake level of 1 mg/day (EFSA, 2006) is exceeded. Unmetabolized FA has been associated with some neoplasia, cognitive damage among seniors due to the masking of vitamin B12 deficiency, and also to the reduced efficacy of anti-folate drugs used to treat rheumatoid arthritis or psoriasis (Crider, Bailey, & Berry, 2011; ESCO, 2009).

Considering the importance of FA for human health, its vulnerability to external agents and the potential risks associated with excessive intake, FA encapsulation could be an opportunity to diminish its degradation and improve its bioavailability. Diverse micro- and nanoencapsulation systems have been recently

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reported for folates fortification based on organic encapsulation supports (Aceituno-Medina, Mendoza, Lagaron, & López-Rubio, 2015; Bakhshi, Nangrejo, Stride, & Edirisinghe, 2013; Madziva, Kailasapathy, & Phillips, 2006; Shrestha, Arcot, & Yuliani, 2012; Tomiuk et al., 2012). Some proposed systems have improved the stability of this vitamin in different food matrices and food processing. However, these systems present some instability during processing or ingestion processes, a poor ability to control the FA rate release or provide targeted delivery (Pérez-Esteve, Ruiz-Rico, Martínez-Máñez, & Barat, 2015). As an alternative, inorganic encapsulation systems, such as mesoporous silica particles (MSPs), can be useful in food fortification thanks to large loading capacity, biocompatibility, stability during digestion conditions and controlled release capability (Aznar et al., 2016; Li, Barnes, Bosoy, Stoddart, & Zink, 2012; Pérez-Esteve et al., 2016; Slowing, Vivero-Escoto, Wu, & Lin, 2008; Song & Yang, 2015). Despite some toxicological limitations need to be overcome before starting to use MSP as smart delivery systems in food industry, several studies have assessed the impact of MSPs. Diverse and contradictory results have been observed in some cells or animals treated with MSPs, but the use of functionalized mesoporous silica microparticles seems a good strategy to minimize the risks associated with using MSPs as supports to develop smart delivery systems (Pérez-Esteve, Ruiz-Rico et al., 2015).

In this scenario, we previously reported the design and synthesis of a smart FA delivery system capable of controlling and modifying FA release in different digestion steps (Pérez-Esteve, Fuentes et al., 2015). As a step forward, the present work aimed to evaluate the protective effect of MSPs in relation to FA stability under real food industry conditions. To accomplish this goal, the bioaccessibility and stability of FA encapsulated into a MCM-41 silica support functionalized with amines that acted as molecular gates was investigated after its incorporation into fruit juices. Apple and orange juices were selected as model food systems for their low pH, which should hinder the delivery of the vitamin, and because different amounts of protective active ingredients were present, such as ascorbic acid. In order to establish the influence of MSPs encapsulation, the stability of free and entrapped FA against processing and storage agents, such as high temperature, light and juice composition, was investigated. As far as we know, this is the first study dealing with the protective effect of MSPs on the stability of biomolecules in real food systems.

2. Materials and methods

2.1. Chemicals

Tetraethylorthosilicate (TEOS), *N*-cetyltrimethylammonium bromide (CTABr), sodium hydroxide (NaOH), triethanolamine ($TEAH_3$), *N*-(3-trimethoxysilylpropyl)diethylenetriamine (N3) and phosphoric acid were provided by Sigma-Aldrich (Madrid, Spain). FA was purchased from Schircks Laboratories (Jona, Switzerland). Acetonitrile HPLC grade was provided by Scharlab (Barcelona, Spain). Two fruit juice types (apple and orange) were purchased from local supermarkets. The composition of these juices is shown in Table 1. HPLC analysis revealed a concentration of folic acid below the limit of quantification in both juices. Juices were stored at 4 °C until analyzed.

Table 1
Main nutrients and pH values from apple and orange juices.

	Apple juice	Orange juice
Carbohydrates (g/100 mL)	11.3	9.9
Proteins (g/100 mL)	0.1	0.7
Fats (g/100 mL)	0.1	0.1
Vitamin C (mg/100 mL)	–	40
pH	3.53	3.64

2.2. Synthesis of encapsulated folic acid (*E-FA*)

Synthesis of microparticulated MCM-41 was carried out using CTABr as the structure-directing agent and TEOS as the silica source, and a molar ratio fixed at 7 $TEAH_3$: 2 TEOS:0.52 CTABr:0.5 NaOH:180 H_2O. CTABr was added to a $TEAH_3$ and NaOH solution that contained TEOS at 118 °C. Then water was slowly added, with vigorous stirring at 70 °C. A white suspension was formed after a few minutes of stirring. This mixture was aged in an autoclave at 100 °C for 24 h. The resulting powder was collected by filtration. Then it was washed with distilled water and ethanol and dried at 70 °C. The as-synthesized solid was calcined at 550 °C for 5 h to remove the template phase (Bernardos et al., 2008).

FA was loaded in the calcined MCM-41 microparticles by the impregnation method described by Pérez-Esteve, Fuentes et al. (2015). FA (10 mg/mL) dissolved in phosphate-buffered saline (PBS) was added to 300 mg of MCM-41 in three addition cycles (1.5 mL per cycle). After each addition cycle, the solid was dried at 37 °C to remove water content. After loading and drying, the solid was functionalized with 1.29 mL of N3 in acetate buffer at pH 2. The final mixture was stirred for 5.5 h at room temperature, isolated by vacuum filtration, washed with 300 mL of acetate buffer at pH 2, and dried at room temperature for 24 h.

2.3. Characterization of supports

Powder X-ray diffraction (PXRD), field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), N_2 adsorption-desorption isotherms and zeta potentials were used to characterize the synthesized materials. PXRD was performed in a BrukerD8 Advance diffractometer using CuKα radiation (Bruker, Coventry, UK). FESEM images were acquired with a Zeiss Ultra 55 (Carl Zeiss NTS GmbH, Oberkochen, Germany) and observed in the secondary electron mode. For the TEM analysis, particles were dispersed in dichloromethane and deposited onto copper grids coated with a carbon film (Aname SL, Madrid, Spain). Imaging of the MSPs samples was performed with a JEOL JEM-1010 (JEOL Europe SAS, Croissy-sur-Seine, France) at an acceleration voltage of 80 kV. Single-particle size was estimated by averaging the measured size values of 50 particles. The N_2 adsorption-desorption isotherms were recorded with a Micrometrics ASAP2010 automated sorption analyzer (Micromeritics Instrument Corporation, Norcross, USA). Samples were degassed at 90 °C in vacuum overnight. Specific surface areas were calculated from the adsorption data within the low pressure range by the BET model. Pore size was determined following the BJH method. Zeta potential measurements were taken by a Zetasizer Nano ZS (Malvern Instruments, U.K.). Samples were dispersed in water at a concentration of 1 mg/mL. The zeta potential was calculated from the particle mobility values by applying the Smoluchowski model. Measurements were taken at 25 °C in triplicate.

2.4. Release and bioaccessibility studies

The release kinetics of **E-FA** in fruit juices was performed to assess the capability of polyamines to hinder vitamin release at the juice's natural pH (pH ca. 3.5), and to evaluate if juice composition had any influence on vitamin delivery when the gate was open (pH 7.5). Moreover, the release studies allowed us to calculate the maximum FA release from 1 mg of support in both juices. In a typical experiment, 10 mg of the solid **E-FA** were placed in