Innovative Food Science and Emerging Technologies 29 (2015) 302–307

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

# Encapsulation of curcumin in electrosprayed gelatin microspheres enhances its bioaccessibility and widens its uses in food applications

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ARTICLE INFO

*Article history:*
Received 25 March 2014
Received in revised form 2 March 2015
Accepted 2 March 2015
Available online 19 March 2015

*Keywords:*
Curcumin
Gelatin
Electrohydrodynamic atomization
Bioaccessibility
Antioxidant activity

ABSTRACT

Curcumin has uses as a food colorant and functional ingredient, these uses being restrained owing to its low solubility in water, which limits its dispersion in food matrices and its bioaccessibility. Curcumin–gelatin microparticles produced by electrohydrodynamic atomization were developed to overcome these problems. Microparticles with a size up to 1.2 μm in diameter, in which curcumin was in the amorphous state, were obtained. Both curcumin water solubility and bioaccessibility were significantly improved by encapsulation (38.6 and 11.3-fold higher than commercial curcumin, respectively). A gellified fish product was used to evaluate the coloring capacity of microencapsulated curcumin, finding a better dispersion for microencapsulated curcumin than for commercial one. However, curcumin bioaccessibility was similar owing to curcumin solubilization into the protein matrix. In spite of this, a protective effect of curcumin was observed, as the antioxidant activity of the bioaccessible fraction of the gel supplemented with microencapsulated curcumin was higher.

*Industrial relevance:* Curcumin is a potential natural food coloring and functional ingredient which impairs an attractive yellowish-orange color to food and possesses a wide range of biological activities. However its use in food is restrained owing to its low solubility in water. Curcumin encapsulation using a soluble polymer is a promising strategy to widen the use of curcumin as an ingredient in the food industry.



## 1. Introduction

Nutraceuticals can be incorporated into food systems for the development of functional foods. However, the effectiveness of a functional food depends on the solubility, stability, and bioavailability of the active molecule. Encapsulation may be a means to facilitate the handling and dosing of certain ingredients, additives, or active compounds that pose problems of volatility, stickiness, or low solubility in water, or to release an active compound at controlled rates or under specific conditions (Fang & Bhandari, 2010). In the food industry, encapsulation can also improve the organoleptic characteristics of a product, masking undesirable flavors, odors and colors. Encapsulation can also protect a molecule from degradation or loss of functionality due to the effects of light, oxygen, pH, moisture, or interaction with other food matrix components (de Vos, Faas, Spasojevic, & Sikkema, 2010). Curcumin is a polyphenol found in the rhizomes of the plant *Curcuma longa* that has been traditionally employed as a natural food dye that imparts an attractive bright yellow–orange color. Furthermore, curcumin has been shown to possess antioxidant, anti-inflammatory, antimicrobial, and anti-cancer properties (Ali, Marrif, Noureldayem, Bakheit, & Blunden, 2006). However, there are some problems that restrict the use of curcumin in foods, mainly its low water solubility, limiting not only its solubility/dispersion in food matrices, but also its bioavailability (Wang, Lu, Lv, & Bie, 2009; Yu, Shi, Liu, & Huang, 2012). The bioavailability of a compound that is ingested orally is the fraction of the compound that is absorbed after solubilization in the intestine and that passes to the systemic circulation. Prior to bioavailability studies it is common to assess bioaccessibility, which is defined as the fraction of a compound that is solubilized from a food during gastrointestinal digestion. Thus, bioaccessibility can be considered as a measure of maximum oral bioavailability (Torres-Escribano et al., 2011).

In order to improve functionality of curcumin, several attempts have been made to encapsulate curcumin by several methods, including chemical/physicochemical and physical–mechanical ones (Gomez-Estaca, Balaguer, Gavara, & Hernandez-Munoz, 2012; Mukerjee & Vishwanatha, 2009; Ouyang et al., 2012; Shaikh, Ankola, Beniwal, Singh, & Kumar, 2009; Takahashi, Uechi, Takara, Asikin, & Wada, 2009; Tikekar, Pan, & Nitin, 2013; Wang et al., 2009). The use of electrohydrodynamic atomization (EHDA) or electrospray for the preparation of monodisperse micro/nanoparticles from a multitude of different precursors is well known. The main advantages of EHDA are that it is possible to obtain particles of different diameters with a narrow size distribution, high encapsulation efficiency, and the absence of a tedious separation process to remove particles from solvent, as happens with a great many encapsulation techniques.

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http://dx.doi.org/10.1016/j.ifset.2015.03.004
1466-8564/

In the present work, the biopolymer gelatin was selected to encapsulate curcumin. Owing to the high solubility of gelatin in water, it was expected to be a good carrier to improve the solubility of curcumin in aqueous media. Gelatin is the denatured derivative of collagen, and it consists of a pool of protein fragments of different molecular weights (Gomez-Estaca, Montero, Fernandez-Martin, & Gomez-Guillen, 2009). In the food industry, gelatin is employed to improve the functional properties of foods by improving their elasticity, consistency, and stability (Djagny, Wang, & Xu, 2001); owing to its good film-forming properties it has been used to develop edible films and coatings (Gomez-Estaca et al., 2009); and it has also been used to encapsulate functional ingredients. Specifically, a paper has been published on microencapsulation of curcumin by spray drying using gelatin and porous starch as wall materials, in which curcumin solubility was highly improved (Wang et al., 2009). Other authors also succeeded increasing curcumin solubility, stability, and/or bioactivity by encapsulation (Pan, Zhong, & Baek, 2013; Tikekar et al., 2013). It has recently been reported that the bioavailability of curcumin has been improved by encapsulation in several matrices (Khalil et al., 2012; Ouyang et al., 2012; Shaikh et al., 2009; Takahashi et al., 2009; Tsai et al., 2011; Vitaglione et al., 2012; Yu et al., 2012).

The objectives of the present work have been to increase curcumin solubility in aqueous media in order to broaden its application as a food colorant and to increase its bioaccessibility, by means of microencapsulation in gelatin microparticles produced by electrohydrodynamic atomization.

## 2. Materials and methods

### 2.1. Raw materials

Gelatin from pigskin G-2500 (gel strength ~300 g Bloom), curcumin from *C. longa* (turmeric), and all reagents other than ethanol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethanol was acquired from Panreac (Barcelona, Spain).

### 2.2. Microparticle production

Curcumin (250 mg) was dissolved in 25 mL of 96% (w/w) ethanol by continuous stirring at 60 °C for 10 min. Then 12.5 mL of distilled water, 12.5 mL of acetic acid and 2.5 g of gelatin were added and the mixture was stirred for 30 min at 40 °C. The solution was left to stand at room temperature for 20 min and was then subjected to electrohydrodynamic atomization. The gelatin/curcumin solution was prepared daily and protected from light during the entire process. On this basis, the gelatin concentration was 5 g/100 mL and the curcumin content was 10 g/100 g gelatin.

The experimental set-up used for electrohydrodynamic atomization was supplied by YFLOW Ltd (Málaga, Spain) and it consisted of a stainless steel needle charged by a high voltage power supply. The collector plate was fixed at a working distance of 10 cm below the needle tip and connected to the grounded counter electrode of the power supply. A 5 mL plastic syringe was filled with the solution and a syringe pump was used to control the flow rate at which the solution was dispensed. The syringe outlet was connected to the needle by a Teflon® pipe. The electrospray droplets were dried during the fly time, on the way to the surface of the collector plate, which was previously covered with aluminum foil. The voltage was 14 kV. Two flow rates were assayed, 0.15 and 0.5 mL/h. The relative humidity and temperature of the chamber were set at 30% and 25 °C, respectively. Samples were stored at 0% RH, in the dark, until they were analyzed.

### 2.3. Microparticle morphology

The particle morphology was studied by scanning electron microscopy, using a HITACHI S-4100 unit equipped with a BSE AUTRATA detector and an EMIP 3.0 image capture system (HITACHI, Madrid, Spain). The samples were collected on an aluminum sample holder, which was placed on the surface of the collector plate. The samples were kept at 0% RH, in the dark, and treated with gold–palladium immediately prior to analysis. Images were captured at 10 kV, at a distance of 5 cm, with 20000× or 40000× magnification.

### 2.4. Encapsulation efficiency

Encapsulation efficiency was obtained as the mass ratio between encapsulated curcumin and that used in the preparation of the nanoparticles. The curcumin loaded in the nanoparticles was measured by dissolving the nanoparticles in 80% (w/w) aqueous ethanol and measuring the absorbance of curcumin at 428 nm UV-Visible spectrophotometer. Standard calibration curve for curcumin was prepared in 80% (w/w) ethanol.

### 2.5. Solid state characterization

X-ray powder diffractometry was carried out to investigate the nature of the curcumin loaded in the gelatin microparticles. The XRD patterns of commercial curcumin, curcumin-loaded gelatin microparticles, and gelatin were recorded using a Bruker AXS D500 spectrometer with a Bragg–Brentano geometry at a wavelength of 1.5406 (corresponding to the $Cu_{k\alpha}$ peak). Powder X-ray diffractograms were recorded in a diffraction angle (2θ) range of 2°–40° using a step size of 0.03° and an exposure time of 8 s. The samples were stored for 3 months at 0% RH and at 23 °C in the dark before testing.

### 2.6. Curcumin solubility in water

Curcumin solubility studies were based on the work by Donsi, Wang, Li, and Huang (2010). In triplicate, an accurately weighed amount of 10 mg of commercial curcumin or 100 mg of encapsulated curcumin (containing 10 mg of curcumin), was dissolved in 10 mL of distilled water. The mixture was warmed and stirred at 25 °C for 20 h prior to centrifuging at 4500 ×g (~5000 rpm) for 10 min at room temperature. The absorbance of the supernatant was measured with a UV–Visible spectrophotometer and the curcumin concentration (expressed as mg curcumin/L) was calculated from a previously prepared standard calibration curve of curcumin in 80% (w/w) ethanol.

### 2.7. Application to a gellified fish product

The coloring capacity of the gelatin microparticles incorporating curcumin was assayed on a gellified fish product, which was used as an aqueous food model. Fresh hake (*Merluccius merluccius*) obtained at a local market was headed, gutted, skinned, washed with tap water, and filleted. The chopped muscle was homogenized in a Moulinex® mixer with NaCl (2 g/100 g of mince). Water was also added in the form of ice to obtain a final water content of 83 g/100 g of mince. Commercial curcumin (0.05 g/100 g of mince) or 10% curcumin-loaded gelatin microparticles (0.5 g/100 g of mince) were added and homogenized, so that the final curcumin content for both was 0.05 g/100 g. A control batch without curcumin was also prepared. The fish minces were placed in hermetic polypropylene containers 3 cm in diameter and heat-induced gels were obtained by heating the containers at 90 °C for 50 min in a thermostatic bath.

### 2.8. Color properties

The color of both the microparticles and the fish gels was determined with a Konica Minolta CM-35000d spectrophotometer set to D65 illuminant/10° observer using a 3 mm mask. The CIELAB color space was used to determine the parameters: L* [black (0) to white (100)], a* [greenness (−) to redness (+)] and b* [blueness (−) to