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Fabrication of amorphous curcumin nanosuspensions using β-lactoglobulin to enhance solubility, stability, and bioavailability

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ABSTRACT

Curcumin has low aqueous stability and solubility in its native form. It also has a low bioavailability which presents a major barrier to its use in fortifying food products. The aim of this work was to reduce the size of curcumin crystals to the nanoscale and subsequently stabilize them in an amorphous form. To this end, amorphous curcumin nanosuspensions were fabricated using the antisolvent precipitation method with β-lactoglobulin (β-lg) as a stabilizer. The resulting amorphous curcumin nanosuspensions were in the size range of 150–175 nm with unimodal size distribution. The curcumin particles were amorphous and were molecularly dispersed within the β-lg as confirmed by differential scanning calorimetry (DSC) and X-ray diffraction (XRD) studies. The solubility of the amorphous curcumin nanosuspension was enhanced ~35-fold due to the reduced size and lower crystallinity. Among the formulations, the amorphous curcumin nanosuspensions stabilized with β-lg and prepared at pH 3.4 (β-lg-cur 3.4), showed maximum aqueous stability which was >90% after 30 days. An *in vitro* study using Caco-2 cell lines showed a significant increase in curcumin bioavailability after stabilization with β-lg.

1. Introduction

In recent years, the food industry has shown an increased desire to incorporate nutraceuticals with properties beneficial to health into food products. Curcumin (diferuloylmethane) is one such yellow hydrophobic polyphenol derived from the herb turmeric (*Curcuma longa*) [1]. Unfortunately, this compound cannot be readily incorporated into the food products due to its aqueous insolubility which leads to its degradation and trifling bioavailability [1,2].

In the past, several attempts have been made to improve the stability and bioavailability of curcumin using various types of delivery systems and carrier materials (lipids, polymers, carbohydrates, *etc.*) [3–6]. Unfortunately their application in food matrix is compromised or minimal due to the factors like toxicity of the delivery systems *e.g.* carrier material, surfactants, *etc.*, uncorroborated cost to benefit ratio, difficulty in availability of raw materials with required quality, quantity, *etc.* [7]. Overall, it would be useful if a system could be developed which increases solubility and bioavailability of curcumin which is specifically suitable to use in food products. In this regard recently very few efforts have been made to fabricate curcumin–β-cyclodextrin complex [8,9] and protein nanoparticles [10] to use these nanocarriers in food products. However, further research is highly warranted to discover new types of carrier materials and methods to increase solubility and stability of curcumin in order to fortify their application in food, pharma and cosmetic application.

In this observe, nanonization technique has been used to produce nanosized curcumin particles with an intention of increasing curcumin solubility, stability and bioavailability. To achieve nanonization, antisolvent precipitation method which is a well-accepted method for the production of food-grade nanoparticles and crystals was chosen [11]. However during the antisolvent precipitation, newly formed nanoparticles used to have increased surface Gibbs free (δG) energy and particle growth tendency by coagulation and condensation which leads to recrystalization. This recrystalization processes compromise the advantage of higher saturation solubility and bioavailability, and faster dissolution rate associated with amorphous state of molecules in comparison to crystalline state of the same molecule [12]. Curcumin which exists in amorphous form initially after precipitation, gets converted into large crystals as time proceeds due to aggregation [13]. Hence to get the maximum solubility and bioavailability, it is necessary to prevent the recrystalization process.

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In this regard, to avoid the particle aggregation by reducing the interfacial tension, selection of suitable stabilizer is of utmost importance [14]. In this stair, food proteins are well suited for the desired application due to their non-toxicity and safe history of human consumption. Above or below the isoelectric point, proteins exhibit amphiphilic nature and hence they act as an efficient surfactant. In addition, recent studies have shown to increase the stability, solubility and antioxidant activity of curcumin when either entrapped or after binding with food proteins such as β-lg and bovine serum albumin [15,16]. Further, due to β-lg stability in acidic pH and the presence of β-lg specific receptors in small intestine which will facilitate to achieve our goal of increasing the oral bioavailability of curcumin, β-lg has been selected over other proteins as a stabilizer [17].

Therefore the objective of the study was to: (a) fabricate an amorphous curcumin nanosuspension, using β-lg as stabilizer, (b) study the stability of these nanosuspensions in a model beverage system, and (c) study the *in vitro* bioavailability of the nanosuspensions using Caco-2 cell lines. This study is novel because of the fabrication of amorphous curcumin nanosuspensions and the avoidance of undesired recrystalization by molecularly dispersing the nanosuspension within β-lg. For the first time we have applied nanonization technique to incorporate bioactive compounds into liquid foods.

2. Materials and methods

2.1. Materials

Curcumin (>95% pure) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Protein β-lg was procured from Davisco Foods International, Inc. (Le Sueur, MN, USA). The protein content was 98.0% and 93.4% on a dry basis and as a percentage of total weight, respectively. The fat, ash, and moisture contents were reported to be 0.1%, 1.9%, and 4.7%, respectively. All other chemicals were of HPLC or analytical grade.

2.2. Preparation of β-lg stabilized amorphous curcumin nanosuspensions

Solutions of antisolvent consisting of water and a hydrophilic surfactant (β-lg; native or denatured; 2 mg/mL) with different pH values (3.4, 5.4 and 7.04) were prepared. Denaturation was done to expose the hydrophobic moieties of the β-lg and pH was adjusted to acidic and basic pH to induce conformational changes in the β-lg. In earlier studies, changes in the pH and heat induced transformation in the basic protein structure are known to affect the curcumin binding with β-lg [15,16]. Sodium azide aqueous solution (0.002%, w/w) was added to the β-lg solution to avoid the bacterial contamination. Both original and denatured β-lg solutions were stored at <4 °C for maximum 7 days. The solvent phase was prepared by dissolving curcumin in ethanol (4.0 mg/mL).

To begin with, both solvent and antisolvent phases were cooled below 4 °C and then the solvent phase was added to the antisolvent phase under magnetic stirring. The resulting nanosuspension was further processed using a probe-type ultrasonicator (SONI-1 VCX130, Sonics and Materials Inc., Newton, CT, USA). Finally, the solvent was evaporated under reduced pressure at 30 °C using a rotary evaporator. For the production of amorphous curcumin nanosuspensions, the various processing parameters that were studied were stirring speed (200–1500 rpm), temperature (5–25 °C), sonication power (20–40%) and solvent to antisolvent ratio (1:10–1:30). Freshly prepared samples were lyophilized using 1% trehelose as a cryoprotectant. All samples were produced in at least triplicate.

2.3. Particle size, zeta potential and morphology analysis

The average particle diameter, size distribution, and zeta potential (surface charge) of the nanosuspensions were measured, after suitable dilution, using a zeta-potential and particle size analyzer (DelsaNano, Beckman Coulter, Inc., Fullerton, CA, USA) at 25 °C with a scattering angle of 165°. Both the size and surface charge measurements were performed in at least triplicate ($n \geq 3$) and the reported results are the means of the readings.

The morphology of the lyophilized samples of curcumin nanosuspension was studied using a low vacuum scanning electron microscope (S-350 N, Hitachi Science System, Ltd., Ibaraki, Japan). Each sample was fixed on the surface of a V1 mica disk (20 mm diameter) of the highest quality grade using double-sided tape and then gold-coated under vacuum using a sputter coater. Finally, observations were made under an accelerating voltage of 15.0 kV.

2.4. Differential scanning calorimetry (DSC)

The crystallinity profiles of the samples were determined using DSC (DSC 200 F3, Netzsch-Gerätebau GmbH, Selb, Germany). For the DSC measurements ~4 mg of freeze dried samples were weighed accurately into a standard aluminum pan and then hermetically sealed with a standard aluminum lid. An empty aluminum pan served as a reference. All samples were heated from 25 °C to 90 °C at a heating rate of 10 °C/min.

2.5. X-ray diffraction (XRD)

Powder X-ray diffraction (PXRD) studies of the raw curcumin and lyophilized powders of curcumin nanosuspension, curcumin nanosuspension stabilized by native and denatured β-lg at different pH values (3.4 and 7.04) and curcumin nanosuspension without any stabilizers were carried out using a high resolution X-ray diffractometer (X'Pert PRO MPD, PANalytical, Almelo, Netherlands). The X-ray diffractometer was operated at a scan rate of 4 °C/min for 2 h between 5° and 40°.

2.6. Short term stability study

Freshly prepared curcumin nanosuspension formulations stabilized with native β-lactoglobulin (β-lg) or denatured β-lactoglobulin (Dβ-lg) fabricated at different pH (3.4 and 7.04) *i.e.* (β-lg-cur 3.4, β-lg-cur 7.04, Dβ-lg-cur 3.4, and Dβ-lg-cur 7.04) and a curcumin nanosuspension without β-lg were selected for short-term stability studies. The stability of curcumin nanosuspensions was assessed by tightly sealing them in glass containers and keeping them at 4 °C for 1 month. The mean particle size and curcumin content were considered to be indicators of formulation stability and were evaluated at predetermined time intervals (0, 7, 15, and 30 days) using HPLC as described previously [1]. Briefly, an Agilent-1200 HPLC System controlled by Chem Station software (Hewlett-Packed, Wilmington, DE, USA) equipped with an analytical C18 column (Zorbax Eclipse XDB-C18, 4.6 mm × 150 mm, 5 μm packing) was used for the detection of curcumin. The mobile phase consisted of methanol, acetonitrile, and 5% acetic acid at a ratio of 35:55:10 (v/v). The flow rate was 0.8 mL/min. The stability of curcumin was calculated using the formula:

$$\text{Curcumin stability}\,(\%) = \frac{\text{curcumin detected}}{\text{initial curcumin added}} \times 100$$