Food Chemistry 268 (2018) 504–512

Contents lists available at ScienceDirect

Food Chemistry

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

Modification of soy protein isolate by glutaminase for nanocomplexation with curcumin

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ARTICLE INFO

Keywords:
Soy protein isolate
Glutaminase
Curcumin
Complexation
Functional properties

ABSTRACT

Soy protein isolate (SPI) was first treated with glutaminase to yield modified SPI (E-SPI) before its complexation with curcumin. Comparisons were made between SPI and E-SPI concerning their characteristics and effectiveness in complexing with curcumin, along with changes in physicochemical properties, 2,2-diphenyl-1-picryhydrazyl (DPPH·)-scavenging capacity and storage stability of curcumin upon complexation. The action of glutaminase did not markedly change SPI structure, but modified some secondary structures, causing greater protein unfolding with more hydrophobic clusters and amino acids exposed. Glutaminase treatment increased DPPH·-scavenging capacity, foaming capacity and stability of SPI. The protein concentration played a role in the changes induced by glutaminase treatment and complexation with curcumin, and 1% protein seemed beneficial (highest DPPH scavenging activity; highest loading amount 10.07%; 98% and > 90% curcumin retained in 1%-E-SPI at 4 °C for 2 h and 12 h, respectively). Therefore, glutaminase treatment of SPI before complexation with curcumin appeared to be appropriate.

1. Introduction

Curcumin is a natural low molecular-weight phenolic compound obtainable from the rhizomes of turmeric (*Curcuma longa Linn.*), and has been widely used as a food colouring agent owing to its intense yellow colour (Dai et al., 2017). Curcumin exhibits a wide range of bioactivities, including anti-oxidative, anti-cancer, anti-inflammatory, anti-ulcer and antimicrobial effects (Tapal & Tiku, 2012). However, the application of this compound as a bioactive ingredient in food and pharmaceutical formulations is limited, due to its low solubility, stability, dissolution rate in the gastrointestinal tract and bioavailability (Anand, Kunnumakkara, Newman, & Aggarwal, 2007). A number of efficient encapsulation techniques and approaches have been proposed to deliver curcumin; these include nanocomplexes (Mirpoor, Hosseini, & Yousefi, 2017), nanoparticles (Fan, Yi, Zhang, & Yokoyama, 2018), nano-emulsions (Lahiri, Ranoo, Zaibudeen, & Philip, 2017), liposomes and micelles (Chen, Li, & Tang, 2015b). The use of curcumin-containing complexes is a popular approach, owing to the advantages of complexation, such as relatively high solubility and stability in solutions. The materials used for such delivery systems, based on complexation, include polysaccharides (Chen, Ou, Chen, & Tang, 2017) and proteins, with the latter encompassing animal proteins, such as bovine serum albumin (Fan et al., 2018), whey proteins (Dhillon et al., 2017) and vegetable proteins, such as soy protein (Pujara, Jambhrunkar, Wong, McGuckin, & Popat, 2017).

Soy protein isolate (SPI), exhibits desirable processing and nutritional properties, and has been widely used in food applications (Mateos-Aparicio, Cuenca, Villanueva-Suarez, & Zapata-Revilla, 2008; Pizones Ruiz-Henestrosa et al., 2007);). SPI contains mainly glycinin (MW = 360,000, ~60%) and β-conglycinin (MW = 180,000, ~40%), and has abundant and balanced proportions of polar, nonpolar and charge amino acids (Teng et al., 2009). In aqueous solutions, the proteins in SPI can not only form globular structures containing a hydrophilic outer layer and hydrophobic centre, but also generate small water-soluble aggregates (especially in the presence of solvents or crosslinking agents) (Lazko, Popineau, & Legrand, 2004; Liu, Tian, Zeng, & Chang, 2008). Recently, considerable attention has been paid to soy protein aggregate particles. Protein nanoparticles are prepared by monitoring the attractive and repulsive forces within the protein to increase protein unfolding and reduce intramolecular hydrophobic interactions (Ko & Gunasekaran, 2006). Different approaches have been used to prepare SPI particles, including heating (Chen, Li, & Tang, 2015a), ultrasonic treatment (Chen et al., 2015b), microwaving (Zhang et al., 2017), addition of solvents or crossing-linking agents (Teng, Luo, & Wang, 2012) and cold-gelation (Zhang, Field, Vine, & Chen, 2015). However, these approaches may lead to denaturation of soy protein and

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https://doi.org/10.1016/j.foodchem.2018.06.059
Received 10 January 2018; Received in revised form 20 May 2018; Accepted 12 June 2018
Available online 15 June 2018

even introduction of organic matters, causing decreased food quality and loss of nutritional value. Appropriate pretreatment(s) can generate soy protein aggregate particles, such as those using acid(s), alkaline(s) and/or enzyme(s). Since acids and alkalies tend to cause undesired breakage of molecular bonds and/or protein structure, an enzymatic treatment, featured in its simple process and mild reaction conditions, has become a common practice. Several enzymes have been used for this purpose, including protease (Chen, Li, Xu, Sun, & Leng, 2017), hydrolase (Kramer, Jurrius, deRijk, Derksen, & Cuperus, 1997) and protein-glutaminase (Suppavorasatit, Lee, & Cadwallader, 2013). Even though quite a few studies have been conducted on protein-bioactive complexes, there has been very little research reported on enzymatic protein-curcumin complexes, except for that formed with enzymatic soy protein hydrolysate by papain (Feng et al., 2015).

This study aimed to expand the concept of "enzymatic protein-curcumin complexes" and explored novel approaches for producing such complexes. In recent years, protein-glutaminase, which can deamidate proteins and improve the solubility of proteins, is gaining increasing attention. Li and Zhao (2012) treated soy protein with glutaminase for more than 24 h (so-called "limited deamidation") to modify soy protein in order to achieve desired characteristics, such as processing properties, including solubility, viscosity and chelating activity. In this study, an enzymatic treatment of soy protein with glutaminase was used to modify soy protein isolate to yield "E-SPI" with desirable properties for encapsulating curcumin. Fourier transform infrared spectroscopy (FT-IR) was used to examine the structure of E-SPI and its complex with curcumin, as compared with SPI and its complex. Fluorescence emission spectra of SPI and E-SPI, upon addition of curcumin at different concentrations of particles, were obtained to evaluate the binding between E-SPI/SPI particles and curcumin. Comparison was made between SPI-Cur (SPI-curcumin) and E-SPI-Cur (E-SPI-curcumin) complexes concerning the stability, 2,2-diphenyl-1-picryhydrazyl (DPPH) radical-scavenging activity and some functional properties, such as foaming capacity and emulsion ability.

2. Materials and methods

2.1. Materials and chemicals

Curcumin (98% purity) was gifted from Yafu Xingyuan Food Industry Co., Ltd (Zhuhai, Guangdong, China). Glutaminase (EC 3.5.1.2) was purchased from Amano Company (Japan). Defatted soy flour was purchased from Shandong Yuwang Industrial and Commercial Co. Ltd. (Shandong province, China). Soy bean oil (Arawana Brand) and corn oil (Arawana Brand) were both purchased from the supermarket (Guangzhou, Guangdong, China). 1-Anilinonaphthalene-8-sulfonic acid (ANS^-) and DPPH were both purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Preparation of soy protein isolate (SPI) and enzymatically modified soy protein isolate (E-SPI)

2.2.1. Preparation of soy protein isolate (SPI)

SPI was prepared via alkali extraction and acid precipitation, following the method of Teng, Luo, and Wang (2013) with slight modification. Defatted soy flour was dispersed in a 10-fold volume (w/w) of distilled water, before pH adjustment to 8.0 with 2 M NaOH. After a 4 h extraction under vigorous stirring with an overhead stirrer (Shanghai Jiuran Instrument Equipment Co., Ltd, Shanghai, China), the solution was centrifuged (8818 × *g*, 20 min, 4 °C), using a refrigerated centrifuge (GL-23M, Changsha Xiangzhi centrifuge CO., Ltd, Changsha, China). The obtained supernatant was allowed to stand at 4 °C for 30 min after the pH was adjusted to 4.5 with 2 M HCl. Then centrifugation (8818 × *g*, 4 °C, 25 min) was applied to collect the insoluble fraction ("precipitate"). The precipitate was washed 3 times with distilled water before being re-dissolved with 2 M NaOH. After full dissolution, the protein solution was dialyzed against distilled water at 4 °C for 48 h with water changed every 6 h. The dialyzed solution was freeze-dried, using a Scientz-18N freeze dryer (Ningbo Scientz Biotechnology CO., Ltd, Ningbo, China) to yield "soy protein isolate" (SPI, with a protein content of 90.2% (dried basis) determined by the Kjeldahl method of nitrogen analysis) (Shanghai Fiber Inspection Instrument Co., Ltd, Shanghai, China). The freeze-dried SPI was stored in sealed pouches at 25 °C before further use.

2.2.2. Modification of soy protein isolate by glutaminase to yield E-SPI

Optimal conditions for the modification of SPI using glutaminase were determined, using response surface analysis (Fig. S1). Under the optimal condition, the best foaming characteristics of E-SPI were achieved. The general procedure of such an enzymatic modification was as follows: the freeze-dried SPI (1 g, dried basis) obtained from Section 2.2.1 was re-dissolved in 100 ml of distilled water to give a protein concentration of 1% (w/v), before pH adjustment to 8.5 using 2 M NaOH with magnetic stirring for 30 min. Upon addition of 4 U/g (protein) of glutaminase, the solution was incubated in a water bath at 45 °C for 1 h and then at 85 °C for 10 min to inactivate the enzyme. After cooling to room temperature, the pH of the reaction mixture was adjusted to 7.0 with the 2 M HCl and freeze dried. The freeze-dried sample "E-SPI" was stored in sealed pouches at 25 °C before further use.

2.3. Complexation of curcumin with soy protein isolate

2.3.1. Preparation of complexes

The SPI and E-SPI stock solutions (8% w/v) were prepared by dissolving corresponding freeze-dried powder in distilled water, and one half of each type of stock solution was then diluted with distilled water, to give stock solutions with different concentrations in the range of 1%-8% (such as 1% SPI, 8% SPI, 1% E-SPI, 8% E-SPI). In parallel, curcumin stock solution (0–5.5 mg/ml) was prepared by dissolving curcumin in absolute ethanol. Then each of the SPI or E-SPI stock solutions was mixed with a curcumin ethanol solution at a volume ratio of 5:1 under stirring for 30 min. All these mixtures were centrifuged at 3000 × *g* and 4 °C for 20 min (to remove insoluble residues) before freeze-drying. The freeze-dried samples were stored in sealed pouches at 25 °C before further evaluation.

Solutions of SPI/E-SPI and curcumin complexes, with protein concentrations of 5%, 6%, 7% and 8%, were used for turbidity evaluation. The light absorbance of SPI, E-SPI or their complexes with curcumin was determined spectrophotometrically at 600 nm, using a UV–Vis spectrophotometer (UV765, Shanghai Youke Instrument co. Ltd, Shanghai, China).

2.3.2. Determination of encapsulation efficiency (EE) and loading amount (LA)

EE and LA were determined using the method described by Chen et al. (2015b). EE was defined as the percentage of curcumin in the complexes and evaluated by the following equation:

$$EE\% = \left[1 - \frac{\text{content of free curcummin (mg)}}{\text{Total content of curcumin (mg)}}\right] \times 100$$

The free curcumin content was determined by taking a portion of the precipitate resulting from the centrifugation step in Section 2.3.1. "Preparation of complexes". Then the precipitate was re-dissolved in absolute ethanol (5 ml) under gentle stirring, using a magnetic stirrer for 5 min before centrifugation at 8818 × *g* and 4 °C for 20 min (to collect the supernatant). The supernatant was used to determine the free curcumin content spectrophotometrically at 426 nm, using a UV − Vis spectrophotometer as described above, based on the pre-established curcumin standard curve (R^2 = 0.998). Loading amount (LA) was calculated using the following equation: