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Food Science and Human Wellness 2 (2013) 124–131

Food Science and Human Wellness

www.elsevier.com/locate/fshw

Optimization of genistein solubilization by κ-carrageenan hydrogel using response surface methodology

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Received 28 February 2013; received in revised form 20 May 2013; accepted 4 June 2013

Abstract

κ-Carrageenan was explored to improve genistein solubility by matrix retention. The corresponding maximum value in the efficiency of retention (*Re*) (50.48 mg/100 mg) was achieved when variables were set as: pH 4.76, temperature 52.12 °C and genistein concentration 0.27 mg/mL. The coefficient of determination (R^2) of the response surface regression model presented in this study was 0.9848. The evidences from XRD, DSC and FT-IR attested the amorphous form of genistein in hydrogel matrix. Importantly, the solubility of genistein in hydrogel amorphous form (16.84–34.42 μg/mL) was much higher than that of its free crystalline form (1.89–6.09 μg/mL) over 30–90 °C.

Keywords: Genistein; Carrageenan; Hydrogel matrix; Response surface methodology; Water solubility

1. Introduction

Genistein [4′,5,7-trihydroxyisoflavone (Supplement 1)], the most abundant aglycone form of isoflavone in soybean, is associated with a variety of beneficial health effects. The approved beneficial functions included reducing risk of cardiovascular disease, lowering rates of prostate, breast, and colon cancers [1], and improving bone composition [2,3]. But the application of genistein has been limited by its poor water solubility and bioavailability. Even worse once absorbed in human body, genistein undergoes rapid degradation and excretion within 24 h [4,5].

Genistein is classified in class II (poor soluble/permeable) in the Biopharmaceutical Classification System (BCS) [6]. Especially for this class of substance, solubility enhancement is crucial part of the strategies to improve bioavailability. Many studies have been focused on complexation of genistein with cyclodextrins (CDs) to improve water solubility of genistein. Daruházi et al. [7] reported that genistein formed a supramolecule with both β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD), while it did not form a stable complex with α-cyclodextrin (α-CD). The genistein/γ-CD provided a peak genistein concentration of 27 μg/mL, and the genistein/β-CD was 13 μg/mL, in contrast to the plain genistein alone 3 μg/mL.

Matrix retention is a promising method to solubilize various less-soluble compounds. In this technique, the less-soluble compounds are mixed with a water-soluble carrier through various measures. The commonly used carriers are long-chain polymers, such as carrageenan, polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG) [8]. It has been proved that addition of a small amount (0.10%–0.25%, *m/V*), of hydroxypropyl methylcellulose (HPMC) or PVP resulted in significant enhancement of the aqueous solubility of drug, because 30%–50% of drug molecules were bound to the polymers [9]. Dong and Song [10] made a complex of κ-carrageenan with an insoluble drug, which significantly increased the compound's water solubility from 1–2 μg/mL to 30 μg/mL. Nanosizing the complex, solubility of the less-soluble drug increased to 37 μg/mL.

Abbreviations: BCS, biopharmaceutical classification system; CDs, complexation of genistein with cyclodextrins; PVP, polyvinylpyrrolidone; PEG, polyethylene glycol; HPMC, hydroxypropyl methylcellulose; *Re*, the efficiency of retention; BBD, Box–Behnken design; XRD, X-ray diffraction; DSC, differential scanning calorimetry; FT-IR, Fourier transform infrared; SD, standard deviation; ANOVA, analysis of variance; CCC, critical cooperativity concentration.

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Peer review under responsibility of Beijing Academy of Food Sciences.

Production and hosting by Elsevier

2213-4530
http://dx.doi.org/10.1016/j.fshw.2013.06.001

Tightly related to the present work, hydrocolloids are mostly used to hold water and provide texture for food. They also have the ability to bind guest molecules and by this influence the characteristics of guest molecules. In a broad sense, the term binding includes adsorption and physical entrapment in colloid matrices, as well as inclusion complexation [11]. Genistein also could be absorbed by hydrocolloids. Pandjaitan et al. [12] reported genistein was enriched in soy protein with the addition of hydrocolloids. But no study has applied κ-carrageenan to improve water solubility of genistein.

The goal of this study was to present a novel approach to prepare κ-carrageenan/genistein matrix with an aim to improve water solubility of genistein. Operational parameters were optimized. Additionally, the XRD, DSC and FT-IR were employed to investigate the characteristics of matrix. We hope this study will be helpful to further exploit for utilization of κ-carrageenan hydrogel to improve solubility of poorly water-soluble compounds.

2. Materials and methods

2.1. Materials

Genistein (4′,5,7-trihydroxyisoflavone, $C_{15}H_{10}O_5$, molecular weight 270.24) was obtained from Chengdu Purifa Scientific Ltd. (Chengdu, China), with the purity higher than 98%. Genistein solution was prepared by dissolving in 0.1 mol/L NaOH at 30 °C with genistein concentration ranging from 0.1 mg/mL to 2 mg/mL. κ-Carrageenan was supplied by Tianjing Bodi Chemical Co., Ltd. (Tianjing, China) and employed without any further purification. κ-Carrageenan was dissolved in water (0.2 g, 40 mL) at 80 °C under stirring for 60 min to prepare homogeneous solution and then cooled to desired temperature.

Water used throughout the study was double-distilled, and then filtered through 0.22 μm Millipore® GSWP filters (Bedford, USA). Solutions to be analyzed by HPLC were prior filtered through 0.45 μm Sartorius Minisart®-SRP 15 PTFE filters (Germany). The HPLC-grade methanol was purchased from Tianjing Siyou Fine Chemical Co., Ltd. (Tianjing, China). All other reagents were of analytical grade.

2.2. Preparation of genistein–hydrocolloid complex

Complexation was carried out by the acidification of an alkali solution ($NaOH/H_3PO_4$) as previously described [13,14]. When κ-carrageenan solution reached 50 °C, 10 mL of genistein solution was injected. The final concentrations were κ-carrageenan at 4 mg/mL and genistein at 0.2 mg/mL. After stirring for 20 s, the mixture was precipitated by adjusting the pH to 4.7 (±0.1) by using 5% H_3PO_4 and then held for 60 min under gentle stirring at 50 °C. All samples were then centrifuged (14,000 × *g*, 25 min), the supernatant was discarded, and the precipitate was washed twice with an ethanol/water mixture (50:50 *m/m*) and centrifuged as before. The complexes were then freeze-dried.

The single factor experiments were carried out following the same process as above. Final concentration of genistein varied from 0.02 mg/mL to 0.4 mg/mL by altering the concentration of genistein alkali solution. Incubation temperature varied in the range of 30–90 °C, which was the same as hydrocolloid solution reached before genistein solution was injected. Final pH (3–8) and incubation time (5–90 min) were also investigated.

A physical mixture of genistein and κ-carrageenan in the same ratio as the complex was prepared. Genistein and κ-carrageenan were admixed in an agate mortar and pestled to obtain homogeneous blend.

Under the optimal condition the effects of NaCl concentration (10–100 m mol/L) and ethanol concentration (2%–20%, *V/V*) were assessed.

Table 1
Box–Behnken design matrix (in uncoded level of three variables), experimental data and predicted values for three-level-three-factor response surface analysis.

Number[a]	Temperature (°C)	Genistein concentration (mg/mL)	pH	R_e[b]	
	X_1	X_2	X_3	Experimental[c]	Predicted
1	40	0.10	5	24.40 ± 1.31	23.42
2	70	0.10	5	3.45 ± 0.12	2.65
3	40	0.30	5	44.43 ± 1.94	45.24
4	70	0.30	5	47.72 ± 3.83	48.7
5	40	0.20	4	46.95 ± 2.05	48.16
6	70	0.20	4	24.84 ± 0.94	25.88
7	40	0.20	6	28.87 ± 1.02	27.83
8	70	0.20	6	34.03 ± 1.68	32.82
9	55	0.10	4	15.00 ± 1.28	14.77
10	55	0.30	4	47.96 ± 2.73	45.94
11	55	0.10	6	3.29 ± 0.10	5.31
12	55	0.30	6	41.78 ± 0.22	42.01
13	55	0.20	5	48.03 ± 2.79	46.41
14	55	0.20	5	47.12 ± 2.00	46.41
15	55	0.20	5	43.06 ± 2.21	46.41
16	55	0.20	5	43.15 ± 1.75	46.41
17	55	0.20	5	50.71 ± 2.83	46.41

[a] Experiments were conducted in a random order.
[b] The efficiency of retention of genistein with κ-carrageenan (mg genistein/100 mg κ-carrageenan).
[c] Each value represented the mean ± SD ($n = 3$).

2.3. High performance liquid chromatography (HPLC) to quantify genistein content

Genistein content in the matrix was tested by full dissolution of the matrix in 0.1 N NaOH for the release of genistein [13]. Five milligrams of the matrix was incubated in 1 mL of 0.1 N NaOH solutions at 30 °C for 12 h. Because genistein is poorly soluble in water, after this incubation, samples were diluted by phosphate buffer (PBS) to a concentration below their solubility limit. PBS (159 mL, 20 m mol/L phosphate, pH 6.9, and 10 m mol/L NaCl) was added to the solution. Genistein was quantified from the solution by HPLC. The efficiency of retention (*Re*, mg/100 mg) was used to represent genistein content (mg) in 100 mg complex. An LC-20AD HPLC system (Shimadzu, Japan) equipped with a diode array detector (D-M20A) and auto sampler (SIL-20A) was applied to quantify genistein content. HPLC analysis was carried out on an Ultimate 18C reverse phase column (Thermo) 250 mm × 4 mm with 5 μm packing. Samples were eluted at