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Original Article

Microspheres as carriers for lipase inhibitory substances to reduce dietary triglyceride absorption in mice

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ABSTRACT

The present study intends to use microspheres as a delivery system of chlorogenic acid (CGA) to investigate the influences of CGA microspheres on dietary fat absorption and fecal triglyceride excretion in a mice model. Microspheres have an average particle size of about 53.3 μm. Results indicated that the microspheres were capable of gradually releasing the preloaded CGA into the surrounding medium. Their bioadhesive property might help prolong the gastrointestinal transit time in mice, and render a better mixing and contact between CGA and triglyceride. Consumption of CGA microspheres resulted in a significantly higher level of fecal triglyceride (119–144%) as compared with the corresponding control groups. A microsphere would be a desirable vehicle for CGA to improve its efficacy along the intestine.

1. Introduction

A direct relationship between the incidence of overweight or obesity and the ingestion of dietary triglyceride (TG) has been proved [1,2]. Pancreatic lipase is responsible for the hydrolysis of 50–70 % of total dietary TG to produce free fatty acids and monoglycerides [3,4]. Suppressing TG hydrolysis and absorption by inhibiting lipase activity was thus an effective way to prevent obesity [5,6]. Some natural bioactive compounds such as catechins, tannins, and chlorogenic acids (CGAs) have been reported to show pancreatic lipase-inhibiting activity [6,7]. Their ability to reduce dietary TG hydrolysis could be enhanced by immobilizing those bioactive ingredients with different carriers [8–10].

Carriers such as microspheres, nanoparticles, and liposomes might offer smart approaches for the delivery of active ingredients by immobilizing them to the carrier particle and regulating their release and absorption [8]. The release of a delivery system is affected mainly by the microstructure and material properties of carriers [10]. Microspheres were believed to be potential carriers for drugs, cell delivery, and

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tissue regeneration as a scaffold [9]. Their ability to be widely distributed throughout the gastrointestinal tract was considered to be one of the advantages to improve drug absorption and reduce side effects [11].

Making microspheres with materials that offer bioadhesive characteristics would be desirable in rendering the carriers capable of attaching to mucosal surfaces, and hence to prolong the residence time and improve the physiological availability of bioactive ingredients [12]. The presence of hydrophilic groups, such as hydroxyl, carboxyl, and amino groups, in a polysaccharide structure enhances its bioadhesion to biological tissues by forming noncovalent bonds. It was a useful strategy to improve physiological availability of bioactive ingredients [13]. Several studies have introduced the use of some natural polysaccharides such as alginate, starch, and chitosan as carrier materials due to their nontoxic, biodegradable, biocompatible, and bioadhesive properties [14].

The present study intends to use microspheres as a delivery system of CGA. Influences of CGA microspheres on dietary fat absorption and fecal TG excretion were evaluated in a mice model. The microspheres were characterized in terms of morphology, particle size, moisture content, bulk density, and flowability. *In vitro* collapse process and CGA release profile of the CGA-microspherical carriers were also discussed.

2. Methods

2.1. Preparation of microspheres

Microspheres were prepared according to the method described by Anandharamakrishnan et al [15], with some modifications. The feed solution, which was prepared by corn starch (Sigma-Aldrich Corp., St Louis, MO, USA) at a concentration of 5 g in 95 mL of water, was atomized through a two-fluid nozzle. The atomizer was used at a compressed air pressure of 275.8–413.7 kPa. The flow rate of the feed solution was set at 5 mL/min. After the spray-freeze process, the frozen particles were collected and lyophilized. The dried microspheres were stored in a dry cabinet at room temperature for future use.

2.2. Characterization of microspheres

Microspheres were examined by a scanning electron microscope (SEM) for surface morphologies and size distribution (TM-1000; Hitachi High-Technologies Corp., Tokyo, Japan). Size distribution results were analyzed by the particle size distribution analysis software (Mac-View ver.4.0; Mountech Co. Ltd, Tokyo, Japan). Moisture content (g/100 g) was determined according to Association of Official Agricultural Chemists (AOAC) method 934.01 [16]. Bulk density (g/L) and tapped density (g/L) of microspheres were measured according to the method of Chau et al [17], with slight modifications. Tapped density was calculated as mass divided by the final volume of the microsphere sample. Powder flowability was characterized by Hausner ratio, which is calculated by dividing tap bulk density by loose bulk density [18].

2.3. Preparation of CGA microspheres

According to the method described in the "Preparation of microspheres" section, CGA extract (purity 45%), which was obtained from Healthmate Co. Ltd. (Changhua, Taiwan), was added and homogenized with the feeding starch solution at a 4% (w/w) level. After the processes of atomization and drying, dried samples were stored in a dry cabinet at room temperature for further use.

2.4. Determination of CGA content in CGA microspheres

CGA microspheres were stirred in a dissolution media at a ratio of 1:1000 (w/v) on a rotating plate (250 rpm) at 37°C for 60 minutes. Subsequently, the CGA microspheres were homogenized at 10,000 rpm (T18 basic; IKA, Staufen, Germany) for 3 minutes to disrupt the structure of microspheres to release the active ingredient. The mixture was centrifuged at 2400*g* (Universal 320R; Hettich, Tuttlingen, Germany) for 10 minutes. The supernatant was filtered using a 0.45 μm polyvinylidene fluoride (PVDF) syringe filter (Acrodisc, Pall Corporation, Deland, FL, USA). The total CGA contents in the filtrate were determined spectrophotometrically at 324 nm (Genesys 10S UV-Vis; Thermo Fisher Scientific Inc., Waltham, MA, USA) according to AOAC method 957.04 [16].

2.5. Dissolution profiles of microspheres

Microspheres were stirred in a dissolution media at a ratio of 1:50 (w/v) and incubated at 37°C on a rotating plate (100 rpm). At different time points, an aliquot of solution was collected and lyophilized. The dried microsphere samples were observed by a SEM.

2.6. In vitro determination of CGA release

The release of CGA from the CGA microspheres was measured by the method of Bae et al [19]. Briefly, CGA microspheres were mixed with a phosphate buffer (pH 7.2 ± 0.2) at a ratio of 1:50 (w/v), and then stirred at 37°C for 120 minutes on a rotating plate (100 rpm). After being centrifuged at 24*g* for 1 minute, the supernatant was filtered using a 0.45 μm PVDF syringe filter. The content of CGA in the filtrate was analyzed spectrophotometrically at 324 nm by AOAC method 957.04 [16].

2.7. Diets and experimental design

The study protocol was approved by the Animal Care and Use Committee of National Chung Hsing University. Forty-eight male ICR mice (Bltw:CD1, BioLASCO, Taiwan) weighing 29.8 ± 3.6 g were obtained from the National Laboratory Animal Center of Taiwan. Animals were individually housed in a stainless cage in a room maintained at 22 ± 2°C with 12-hour light/dark cycles. Animals were allowed free access to food and water during the experimental period. In the present study, there were six diet groups: one normal chow (NC) control group and five high-fat diet groups, which included high-fat (HF) control, microsphere plus low dose of CGA (CGA-L), microsphere plus high dose of CGA (CGA-H), microsphere