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Curcumin and catechin co-loaded water-in-oil-in-water emulsion and its beverage application

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ABSTRACT

Curcumin and catechin are nutraceuticals and natural health products that have shown several health benefits. However, due to their unstable nature, these compounds cannot be included in food products as such. The prime objective of the current study was to examine the formulation factors that determine the stability of these nutraceuticals and their carrier systems as well as to fabricate water-in-oil-in-water emulsions (W/O/W) to prevent the degradation of both curcumin and catechin in beverage systems. The fabricated emulsion had a volume-weighted mean diameter (d_{43}) of ~4 μm, with an encapsulation efficiency of >90%. Encapsulating the catechin within the inner aqueous phase of the double emulsion increased the stability of catechin by >20% at 23 ± 2 °C and by >40% at 4 °C after 15 days of incubation, as compared to free catechin. In the case of curcumin, >80% was detected after incubation in the beverage system in the form of emulsions, whereas it was reduced to ~40% in the case of free curcumin.

1. Introduction

Natural health products and nutraceutical interventions are currently being investigated on a priority basis for health promotion and disease-risk reduction (Vo & Kim, 2013; Xie, Xia, & Le, 2014). Curcumin a yellow pigment isolated from *Curcuma longa* Linn, and catechin that is mainly composed of epigallocatechin gallate (EGCG), epigallocatechin (EGC) and epicatechin gallate (ECG) are known to be effective bioactive compounds to treat and prevent several diseases like cancer, obesity, infectious disease, and cardiovascular ailments (Aditya, Shim, Yang, Lee, & Ko, 2014; Aditya, Vathsala, Vieira, Murthy, & Souto, 2013; Lv et al., 2014; Nayak, Tiyaboonchai, Patankar, Madhusudhan, & Souto, 2010; Stammler & Volm, 1997). Several studies have shown synergistic increase in the biological activity of curcumin and catechin when used in combination (Manikandan et al., 2012; Xu et al., 2010). Therefore, it will be

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worthwhile to develop food products enriched with these nutraceuticals, especially a combination of both curcumin and catechin. Such curcumin- and catechin-fortified food products can be used to maintain general health in the healthy and can also be used as a nutraceutical to cure diseases (Aditya et al., 2014, 2015).

Unfortunately, these bioactive compounds cannot be readily incorporated into food products because of their unstable nature. Curcumin gets easily degraded in alkaline pH (>7), high temperature, and light. Moreover, because of its hydrophobic nature, it is rapidly eliminated from the body, with little absorption in the gastrointestinal tract (Aditya et al., 2013). Although catechin has greater water solubility than curcumin, its low membrane permeability and low stability in the presence of oxygen, alkaline pH, and high temperature, compromises its bioavailability (Green, Murphy, Schulz, Watkins, & Ferruzzi, 2007). This susceptibility to degradation under conditions that they may encounter before reaching their targeted site of activity reduces their bioactivity. Further, direct addition of these compounds causes undesirable changes in the organoleptic properties of food products, such as off-taste, odor, and appearance. In the last couple of decades, more attention has been paid toward developing delivery systems to overcome the pharmacokinetic mismatch associated with curcumin and catechin and to enable their utilization in the pharmaceutical, food, and cosmetics sectors. Various types of delivery systems such as liposomes, emulsions, lipids, and polymeric and protein nanoparticles, have been evaluated to circumvent their pharmacokinetic mismatch (Aditya et al., 2012; Choi, Aditya, & Ko, 2014; Hamam & Al-Remawi, 2014; Rodrigues et al., 2013). Though the delivery systems are partially successful in overcoming the problem of pharmacokinetic mismatch, most of these systems are limited, in that they are only capable of entrapping either hydrophilic or hydrophobic nutraceuticals at one time. Therefore, when using these delivery systems, it is not possible to get the benefits of the synergistic action of curcumin and catechin. Recently, we successfully fabricated curcumin and catechin co-loaded W/O/W emulsions and observed a significant increase in the stability and bioaccessibility of both these compounds (Aditya et al., 2015). Thus, W/O/W emulsions entrapped with these two synergistically bioactive compounds offer an attractive route to fortify food products with these nutraceuticals and thereby help enhance the nutritional value of such products. In addition, co-loading these compounds allows the reduction in the amount of excipients required to carry the same amount of nutraceutical molecules when loaded individually.

To successfully incorporate W/O/W emulsions, they need to be stable in complex food products and should serve to stabilize curcumin and catechin by preventing degradation in the adverse environments existing both in the food product and within the microstructure of the emulsion.

In the present study, a thorough preformulation evaluation was performed, with the aim of choosing suitable excipients to develop curcumin and catechin co-loaded W/O/W emulsions, suitable for oral intake. The suitability of the fabricated emulsion to increase the stability of curcumin and catechin in a fortified beverage system was also subsequently examined.

2. Materials and methods

2.1. Materials

Catechin and curcumin were purchased from Sigma Aldrich (St. Louis, MO, USA). Olive oil, soybean oil, and sunflower oil were purchased from Daejung Chemicals Ltd. (Seoul, Korea). Tween 80, Span 80, gelatin, and NaCl were also purchased from Sigma Aldrich. Sucrose was purchased from Duksan Pure Chemicals Co., Ltd. (Ansan, Korea). All other chemicals were of analytical grade.

2.2. Selection of emulsifier

The most suitable hydrophobic surfactant to stabilize the water-in-oil (W/O) emulsion was determined by the visual inspection for phase separation and by microscopic evaluation for aggregation and size (Eclipse 80i, Nikon, Tokyo, Japan). Medium chain triglycerol (MCT) was selected as the first oil phase to be studied. MCT was mixed thoroughly with several food-grade hydrophobic emulsifiers such as PGPR (HLB 0.6), Span 80 (HLB 4.3), soy lecithin (HLB 4.1), and Span 80 (90%) + Tween 80 (10%) (HLB 5.37) by stirring at 60 ± 2 °C for at least 15 min. The initial hydrophobic emulsifier concentration used for screening was 8% for all the emulsifiers. Next, distilled water, which constituted the aqueous phase, was also heated to 60 ± 2 °C, added to the oil phase, and homogenized for 8 min at 6000 rpm, using an Ultra-Turrax T25 homogenizer (IKA Labortechnik, Staufeni, Germany). This mixture was sonicated using a probe sonicator (VCX 130, Sonics and Materials Inc., Newtown, CT, USA) at a frequency of 20 kHz and an amplitude of 40% (work time 3 s, rest time 3 s) for 4 min. The volume ratio of the water phase to the oil phase was 25:75 (v/v). The W/O emulsion thus fabricated was stored in a glass bottle and further evaluated for phase separation and aggregation.

2.3. Selection of the oil phase

Compatibility of oil with the hydrophobic emulsifier used is an important factor that determines the stability of primary water in oil (W/O) and secondary (W/O/W) emulsions. Thus W/O emulsions were fabricated by the aforementioned procedure using sunflower oil, olive oil, MCT, or soybean oils, with PGPR (5%) as the hydrophobic surfactant. The volume ratio of the water phase to the oil phase was 25:75. The oil most compatible with PGPR at the tested concentration (5%) was selected based on the viscosity and the emulsion stability index (ESI).

Viscosity was determined as described earlier, with minor modifications in the procedure (Jiao & Burgess, 2003). A controlled-stress rheometer (AR1500ex, TA Instruments, New Castle, DE, USA) with a 40 mm parallel plate was used to carry out the rheological measurements. Viscosity was measured under controlled shear stress ranging from 0.01 to 100 Pa. An oscillation-based procedure at a constant frequency of 1 Hz was used to obtain information on storage and loss moduli at 25 °C. Also, ESI was measured as described earlier (Ahn, Lee, & Kwak, 2013). Briefly, the W/O/W emulsions fabricated were stored in a volumetric flask at 37 °C for 1 h. The volume of the aqueous