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Stability and recovery of cyclodextrin encapsulated catechin in various food matrices

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ABSTRACT

Catechin is astringent in taste, sparingly soluble in water and sensitive to oxygen, light and pH. These properties restrict its application in food products. The present study investigated the stability of inclusion complex (IC) and catechin in various food matrices and investigated *in vitro* recovery profile of catechin and IC in liquid, semi-solid and solid food matrices. Besides, the sensory profile of IC added yogurt was also determined. Results showed that IC and catechin was more stable in solid matrix compared to semi-solid and liquid matrices. IC added in milk and yogurt show the highest percentage recovery of catechin compared to IC added in cheese and catechin added in all the matrices in *in vitro* digestive system. Through IC, β-CD masked the bitterness of catechin. These results suggest that protection of antioxidant such as catechin by β-CD inclusion complex may have applications in functional foods and health supplements.

1. Introduction

Catechin is a flavonoid commonly found in tea, wine, fruit and cacao products. It is an important antioxidant and free radical scavenger (Fan, Sang, & Jiang, 2017; Plumb, de Pascual-Teresa, Santos-Buelga, Cheynier, & Williamson, 1998; Terao, Piskula, & Yao, 1994; Wolfe, Wu, & Liu, 2003). Many research articles have established that *in vitro* studies of catechins show protection against degenerative diseases and a strong inverse relationship between the intake of catechins and risk of CHD mortality (Stein, Keevil, Wiebe, Aeschlimann, & Folts, 1999; Wollny et al., 1999). It has been reported that catechins appear to have greater anti-bacterial activity against Gram positive than Gram negative bacteria (Ikigai, Nakae, Hara, & Shimamura, 1993). Green tea catechins are found to inhibit carcinogenesis of the skin, lung, oesophagus, stomach, liver, small intestine, colon, bladder, prostate, and mammary glands in animal studies Chung et al. (2000). Epigallocatechin gallate (EGCG) has been reported to have many potential targets to act against carcinogens (Huang et al., 1997).

Catechins often exhibit poor bioavailability mainly due to low water solubility and sensitivity to light and heat (Okabe et al., 1999). Moreover, catechins possess an astringent and bitter taste. Thus, it is difficult for them to be incorporated as natural food additives or oral supplements. To circumvent these drawbacks, encapsulation has been investigated as a potential approach.

Cyclodextrins are cyclic oligosaccharides classified by the number of glucopyranose units, commonly 6 (β-CD), 7 (β-CD) and 8 (β-CD). They are naturally occurring and most cyclodextrins are crystalline, homogeneous and non-hygroscopic. Cyclodextrins are biocompatible, generally non-toxic over a wide range of concentrations, relatively inexpensive and produced naturally by enzymatic degradation of starch (Stella & He, 2008). Cyclodextrins have torus-like macro ring shapes with a hydrophobic cavity. They are relatively water-soluble hosts that form inclusion complexes with hydrophobic guests of suitable dimensions (Linde et al., 2010).

The resulting inclusion complexes have found applications in the pharmaceutical and food industries (Ho, Thoo, Young & Siow, 2017a, 2017b; López-Nicolás, Rodríguez-Bonilla, Méndez-Cazorla, & García-Carmona, 2009; López-Nicolás & García-Carmona, 2010; Mangolim et al., 2014; Marcolino, Zanin, Durrant, Benassi, & Matioli, 2011). CDs can be used to control and preserve the natural colour and quality of food (López-Nicolás et al., 2009; López-Nicolás & García-Carmona, 2007). Hydroxypropyl-β-cyclodextrin (HP-β-CD) has been approved by the European Medicine Agency (EMA) and the American Food and Drug Administration (FDA) as safe for both oral and intravenous administration to humans because of its lower toxicity, higher water solubility and stronger inclusion ability than β-cyclodextrin (Gould & Scott, 2005).

The taste of catechins is characterized by bitterness and astringency.

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Masking these properties is important for the development of products that can be consumed regularly. There are number of research papers and patents concerned with the masking of bitterness and astringency using cyclodextrins (Hayashi, Chen, Hiraoka, Ujihara, & Ikezaki, 2010; Okumura, Ichitani, Takihara, & Ko-Ki, 2008). In the pharmaceutical field, inclusion complexation by CDs has been evaluated for many applications, including drug delivery and the taste masking of drugs (Szejtli, 2004).

The structure and composition of the food matrix in which polyphenols are included can either enhance or prevent the recovery and stability of these compounds during digestion and hence, their effectiveness. The co-digestion of polyphenols with different food components, matrices or diets affects their digestibility, bioaccessibility and antioxidant activity (Dupas, Marsset-Baglieri, Ordonaud, Ducept, & Maillard, 2006; McDougall, Dobson, Smith, Blake, & Stewart, 2005; Sengul, Surek, & Nilufer-Erdil, 2014). The recovery of polyphenols from liquid or solid food matrices has also been studied, but mainly in naturally enriched matrices like fruits and juices (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010; Tagliazucchi, Verzelloni, & Conte, 2012). Food matrices have a great impact on recovery and stability of polyphenols. We are unaware of any previous reports of the recovery profile of catechin and its β-cyclodextrin (βCD) inclusion complex (IC) in food matrices. The aim of the present study was to fill this gap, investigating the stability, recovery and sensory profile of catechin and β-CD encapsulated catechin in liquid, semi-solid and solid food matrices, with a view to developing new functional foods and health supplements. This study allows the understanding of storage stability of catechin and the inclusion complex (IC) in different food matrices and the release and retention of both compounds in *in vitro* digestive system. Sensory evaluation of IC added into a food matrix provides an understanding on its sensory attributes.

2. Materials and methods

2.1. Materials

Catechin hydrate, β-CD, HPβ-CD, Mβ-CD, 2,2-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich Inc. (St. Louis, MO). Canola oil, whey protein, sucrose, milk and yogurt were purchased from a local supermarket.

2.2. Inclusion complex (IC) preparation

A mixture of β-CD (0.006 mol) and catechin (0.006 mol) was diluted in 50 ml of water. The mixture was stirred for 5 h in the dark at room temperature and left in the dark for 12 h. The resulting solution was vacuum dried (100 °C) for 10 h and stored at 4 ± 1 °C (Ho et al., 2017a).

2.3. Antioxidant properties

2.3.1. DPPH free radical-scavenging assay

Total antioxidant activity was determined using the DPPH method based on quantification of free radical scavenging activity as described by Chong and Lim (2012). Briefly, 2.0 ml of a freshly prepared solution of DPPH (5.9 mg per 100 ml methanol) was made up to different dilutions in 1.0 ml samples. The mixture was shaken vigorously (vortex) and incubated for 30 min in the dark at room temperature. The absorbance (Abs) of the resulting solution was measured at 517 nm, against a blank of methanol without DPPH and a control without any sample, using a UV–visible spectrophotometer (Lambda 25, Perkin-Elmer, Waltham, MA, USA). The radical scavenging capacity was calculated using the following equation:

$$\text{Inhibition}\,(\%) = [(\text{Abs control} - \text{Abs sample})/\text{Abs control}] \times 100$$

Results were expressed as IC_{50} values calculated by plotting the remaining percentage of DPPH against the sample concentration to obtain the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% (IC_{50} was expressed in μg/ml). The result is reported as AEAC in mg ascorbic acid/g, calculated by $IC_{50\ (ascorbate)}/IC_{50\ (sample)} \times 10^5$.

2.4. Food matrices studies

Stability and recovery of IC in liquid, semi-solid and solid food matrices was studied. Cheese (solid), yogurt (semi-solid) and milk (liquid) were prepared from milk as described below.

2.4.1. Production of cheese

Milk was heated to 31 °C followed by the addition of calcium chloride. Starter culture (Mesophilic direct; Lactococcus lactis ssp. lactis, Lactococcus lactis ssp. cremoris) was then added into the milk and the mixture was allowed to set for 30 min at 32 °C. Rennet was then added and the milk allowed to set again for 45 min until a firm curd was formed. This curd was then sliced into small pieces, set for a further 40 min, heated for another 45 min to 38 °C and maintained at that temperature for 30 min. The curd and whey were ladled into a mold that has been lined with cheesecloth. One teaspoon of salt and either IC or catechin was added into the curd which was then pressed with a 2 kg weight overnight (Fadavi & Beglaryan, 2015).

2.4.2. Production of yogurt

Pasteurized milk (400 ml) was sterilized at 110 °C for 10 min and cooled to 43 °C. Starter culture (yogurt, Lactocillus bulgaricus and Streptococcus thermophiles, 12 ml) was added into the sterilized milk and mixed well. The pH of the solution was monitored to ensure it was approximately 4.6 and the mixture incubated at 43 °C for 24 h (El-Said, Haggag, Fakhr El-Din, Gad, & Farahat, 2014).

2.4.3. Determination of the stability of IC in various food matrices

Approximately 0.1 mg/ml of IC or free catechin were added into cheese (solid), yogurt (semi-solid) and milk (liquid). Food matrices spiked with catechin were stored at 4 °C and aliquots taken weekly for a total of 4 weeks for antioxidant activity determination.

2.4.4. Extraction of catechin from food matrices

The spiked food samples were homogenized with 10 ml of water for 2 min, centrifuged using a refrigerated (4 °C) centrifuge at 10,000*g* for 20 min, the supernatant was collected and the antioxidant activity was measured (Fadavi & Beglaryan, 2015).

2.4.5. In vitro digestion recovery studies

In vitro digestion was simulated using an approach developed by Versantvoort, Oomen, Van de Kamp, Rompelberg, and Sips (2005) with slight modifications. Stimulated saliva was prepared using α-Amylase (≥5 units/mg, glycoprotein with single polypeptide chain of ~475 residues) from human saliva dissolved in phosphate buffer saline (PBS) solution to obtain a stock solution of 90 units/ml, and stored at 20 °C until required. Gastric fluid was prepared with 0.125% (w/v) pepsin (≥250 units/mg), and duodenal fluid was prepared with 0.45% (w/v) pancreatin. Lipase was not added because it is present with co-lipase in porcine pancreatin. IC or catechin (10 mg/g) was added into milk, yogurt and cheese. The cheese was cut into small cubes using a stainless-steel knife with 10 blades spaced 3 mm apart to achieve a constant surface-to-volume ratio of 20 cm^{-1}.

Simulated saliva (pH 6.8) (3 ml) was added to 50 ml centrifuge tubes, each containing either 5 ml of milk, yogurt or 1.5 g of cheese. After incubation with no agitation for 5 min at 37 °C, 6 ml of simulated gastric juice (pH 1.3) was added to each tube. The pH was adjusted to approximately 3 with 5 N HCl, and the mixtures were incubated in a shaking incubator (55 rpm) for 2 h at 37 °C. Following this gastric phase, 6 ml of simulated duodenal juice (pH 8.1) and 1 ml of

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