Food Chemistry 156 (2014) 176–183

Contents lists available at ScienceDirect

Food Chemistry

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

Delivery of green tea catechin and epigallocatechin gallate in liposomes incorporated into low-fat hard cheese

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ARTICLE INFO

Article history:
Received 12 December 2013
Received in revised form 10 January 2014
Accepted 28 January 2014
Available online 6 February 2014

Keywords:
Liposome encapsulation
Catechin
Epigallocatechin gallate
Antioxidants
Cheese
Green tea

ABSTRACT

The encapsulation of green tea catechin and epigallocatechin gallate (EGCG) in soy lecithin liposomes was examined at four concentrations (0%, 0.125%, 0.25% and 0.5% w/v), and inclusion in cheese at 0% and 0.25% w/v. The empty capsules had a mean diameter of 133 nm and significantly ($p < 0.05$) increased with the addition of catechin or EGCG. Electron microscopy revealed the lamellae and central core of the liposomes. Addition of antioxidants gave a significant ($p < 0.05$) increase in the size of liposomes. Liposomes had surface potentials of −42.4 to −46.1 mV with no significant difference between treatments, suggesting stable liposome systems. High efficiency (>70%) and yield (~80%) were achieved from the incorporation of catechin or EGCG inside the liposome structure. Addition of either antioxidant increased the liposome phase transition temperature (>50 °C). Nanocapsules containing these antioxidants were effectively retained within a low-fat hard cheese, presenting a simple and effective delivery vesicle for antioxidants.

1. Introduction

Green tea is obtained from the leaves of the tea plant (*Camilla sinensis*) and produced without further fermentation. This beverage has been consumed worldwide for thousands of years and is an excellent source of health-promoting antioxidant polyphenols (Katiyar & Mukhtar, 1996). Catechins are the main polyphenolic compounds in green tea and are responsible for the majority of the antioxidant activity and bitterness, the most important being (−)-epigallocatechin gallate (EGCG) (Chen & Chan, 1996) which accounts for some of the derived health benefits of green tea (Johnson, Bryant, & Huntley, 2012). Green tea polyphenols have been used as food additives to improve the antioxidant properties and shelf-life of foods (Siripatrawan & Noipha, 2012); however, it can be difficult to retain them effectively in food products with suitable activity, thus necessitating a suitable delivery system. Furthermore, the lability of most green tea catechins at different pH values during food storage (Friedman, Levin, Lee, & Kozukue, 2009) and human digestion (Nakagawa & Miyazawa, 1997), as well as the poor bioavailability (Mukhtar & Ahmad, 2000), have restricted their use as additives in food.

Phenolic compounds can associate with food components, such as proteins, causing significant aggregation and precipitation, and ultimately quantity and/or functional loss of the polyphenols (Bartolome, Estrella, & Hernandez, 2000; Xiao et al., 2011). Encapsulation provides an approach to solve these issues, along with the possibility of increasing the concentration of encapsulated antioxidants within food products (Sun-Waterhouse, Wadhwa, & Waterhouse, 2013). Many encapsulation methods, such as spray drying (Gibis, Vogt, & Weiss, 2012), freeze drying (Laine, Kylli, Heinonen, & Jouppila, 2008), nanoprecipitation (Anand et al., 2010), yeast cells (Shi et al., 2007), emulsions (Di Mattia, Sacchetti, Mastrocola, & Pittia, 2009), and liposomes (Fan, Xu, Xia, & Zhang, 2007; Fang, Hwang, Huang, & Fang, 2006) have been used to protect different polyphenols using a wide range of encapsulating materials. Some disadvantages of these approaches include the use of solvents and non-food grade substances, complicated and expensive equipment, expensive encapsulating materials as well as low encapsulation efficiency, instability of capsules, and large particle sizes that detract from the sensory qualities and appearance of the food product.

Liposomes are an attractive encapsulation system because of their biocompatibility, biodegradability and absence of toxicity, small size, and ability to carry a wide variety of bioactive compounds due to the amphiphilicity of the phospholipid encapsulating material (De Leeuw, de Vijlder, & Bjerring, 2009). Incorporation

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http://dx.doi.org/10.1016/j.foodchem.2014.01.115
0308-8146/

of phenolic compounds into phospholipids, such as soy lecithin, is a novel formulation called "phenolipids", and provides a potential new application for encapsulated phenolics in the food and pharmaceutical industries (Ramadan, 2012). Moreover, the antimicrobial and antiviral activity of phenolic compounds can be increased by soy lecithin (Ramadan, Asker, & Mohamed, 2009).

Two of the major green tea antioxidants, catechin and EGCG, were encapsulated within soy lecithin liposomes, with the aim of protecting these polyphenols from degradation arising from food formulation, processing, storage and digestion. A low-fat hard cheese system was employed as the vehicle for inclusion of liposomes to increase antioxidant activity, with maximum retention of the antioxidants. Although soy lecithin has been explored as a coating material, there are no reports on using a rapid and simple homogenisation process for the encapsulation of catechin or epigallocatechin gallate using this coating material.

2. Material and methods

2.1. Chemicals

(+)-Catechin and EGCG were purchased from Sigma–Aldrich (Auckland, New Zealand) and Sapphire BioScience (Waterloo NSW, Australia), respectively. Soy lecithin was provided by Hawkins Watts (Auckland, New Zealand). Folin–Ciocalteu's phenol reagent was purchased from Merck (Darmstadt, Germany). Methanol (HPLC grade) was from Thermo Fisher Scientific (Auckland, New Zealand). Solutions of 0%, 0.125%, 0.25%, and 0.5% (w/v) of catechin and EGCG were prepared in 0.25 M acetate buffer (pH 3.8).

2.2. Preparation of liposomes

To prepare a coarse liposome suspension, soy lecithin was dispersed in the antioxidant/acetate buffer at a concentration of 1% (w/v) with magnetic stirring for 30 min, followed by homogenisation using a high shear blender (Polytron, PT-MR 2100, Kinematica, Switzerland) at 24,000 rpm (5 × 1 min bursts). Two controls and one blank were set up for comparison. The first control consisted of the antioxidant solutions without lecithin, and were subjected to the same magnetic stirring and homogenisation processes. The second control consisted of soy lecithin dispersed (at 1% w/v) in the same acetate buffer without any added antioxidant, and then stirred and homogenised. The blank consisted of the acetate buffer in the absence of both antioxidants and lecithin, and subjected to the same magnetic stirring and homogenisation processes. The prepared liposomes were stored at 4 °C for 24 h before further characterisation and use.

2.3. Gel filtration

A Sephadex column was prepared using 5% w/w Sephadex G50 (Sigma–Aldrich) in deionised water. Empty 6 ml syringes were placed in collecting tubes and filled with 5 ml of Sephadex suspension. Water was allowed to drain (~5 min) with swirling from the columns until 3 ml of Sephadex gel became available. To expel the excess water from the gel and clean the column, an aliquot (1.5 ml) of the prepared acetate buffer (0.25 M, pH 3.8) was added to the gels. Syringes were placed in capped 15 ml plastic test tubes before being centrifuged at 1207 g, 25 °C for 10 min (Beckman, Brea, CA, USA). Finally, the syringes containing purified Sephadex gels were placed into new 15 ml plastic test tubes, to which 1.5 ml of liposome sample was added, before the centrifugation step was repeated. Gel-filtered liposomes were collected at the bottom of the test tubes and stored at 4 °C for further analysis.

2.4. Particle size and zeta potential

The Z-average, mean diameter and zeta potential of the liposomes were determined using a Zetasizer Nano-ZS series dynamic light scattering instrument (Malvern Instruments, Worchestershire, UK). Each measurement was obtained from the average of three readings of two subsamples. The samples were diluted (1:50) in 0.25 M, pH 3.8 acetate buffer with gentle stirring. The measurements were carried out 24 h after the liposome preparation.

2.5. Structure determination of nanocapsules using electron microscopy

2.5.1. Cryo-scanning electron microscopy

To study the morphology and internal structure of liposomes, cryo-scanning electron microscopy (SEM) was carried out using a field emission scanning electron microscope (JSM 6700-FE SEM, JEOL Ltd., Tokyo, Japan). Prior to observations, the cryo-stage within the microscope was cooled to lower than −140 °C by using a cold nitrogen gas stream. The sample stage in the Gatan Alto 2500 cryo preparation chamber (Gatan Inc, Pleasanton, CA, USA) was cooled to −140 °C using liquid nitrogen and then kept at a constant temperature of −140 °C. For the preparation of test samples, the liposome dispersion was loaded into brass rivets and rapidly frozen in a liquid nitrogen slush, then transferred under vacuum to the preparation chamber. The raised surface of the frozen sample was fractured using a pre-cooled metal blade and then sublimed at a temperature of −100 °C for approximately 2 min to remove ice from the surface. The test samples were then cooled back to −140 °C, sputter coated with 3 nm platinum to increase conductivity, and transferred to the cold stage in the SEM chamber, where it was viewed and imaged at a working distance of 11 mm under an accelerating voltage of 5.0 kV.

2.5.2. Transmission electron microscopy

A freeze fracture electron microscopy (Gradauer et al., 2010) with negative staining was used to reveal the structure of the liposomes. For negative staining, an aliquot (10 μL) of the suspension of loaded liposomes (i.e. containing antioxidants) or empty liposomes was placed on a plasma-glowed, carbon-coated 300 mesh copper grids (ProSciTech Pty. Ltd., Thuringowa, Qld., Australia). Excess sample was blotted with filter paper after 1 min, and 10 μL of 1% phosphotungstic acid (pH 3.8) was immediately applied to the grid and again blotted with filter paper. The grid was air-dried (for a few seconds) before TEM examination. Freeze fracture was carried out by mixing liposomes with 30% glycerol (v/v) followed by freezing in liquid propane and fractured with a Balzers BAF060 freeze-etching apparatus (Balzers, Liechtenstein) under vacuum at −120 °C. Replicas were produced by electron gun deposition of 2 nm of platinum at a 45° angle and 20 nm of carbon at a 90° angle. The coating thickness was controlled using a quartz crystal monitor. The specimens were then thawed and the replicas floated off in distilled water before being mounted on a 300 mesh copper grid and viewed using a Philips CM100 transmission electron microscope at an accelerating voltage of 100 kV (Philips Electron Optics, Eindhoven, The Netherlands). A MegaView 3 digital camera (Soft Imaging System GmBH, Münster, Germany) was used to capture the images.

2.6. Encapsulation efficiency of liposomes

The encapsulation efficiency of liposomes containing either catechin or EGCG was evaluated using the method of Ishii and Nagasaka (2001) with some modifications. This method was based on liposomes being disrupted by ethanol with heating at 70 °C before and after Sephadex gel filtration. Ethanol can disrupt liposomes