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Bioaccessibility of green tea polyphenols incorporated into an edible agar film during simulated human digestion ☆

A.M. López de Lacey [a], B. Giménez [a,*], E. Pérez-Santín [a], R. Faulks [b], G. Mandalari [b,c], M.E. López-Caballero [a], P. Montero [a]

[a] Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN), CSIC**, C/ José Antonio Novais, 10, 28040. Madrid, Spain
[b] Model Gut Platform, Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, United Kingdom
[c] Pharmaco-Biological Department, University of Messina, Vill. SS. Annunziata 98168 Messina, Italy

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ABSTRACT

Simple edible films can be manufactured to meet not only their primary protective purpose but can be easily manipulated to meet sensory expectations and to contain compounds which enhance the protective properties or even have the potential to deliver health benefits. However, the use of such edible films not only to protect the food but as a vehicle to deliver health benefits has not been investigated. In this paper we study agar films containing an aqueous extract of green tea, rich in polyphenol compounds, and the bioaccessibility of these compounds during simulated digestion in the upper gastro-intestinal tract using a dynamic gastric model (DGM) and a static duodenal model. It is concluded that the recovery of the tea compounds added to the agar film mainly occurs in the stomach (50–80%) and that little or no additional recovery is observed in the duodenum. Furthermore, the green tea compounds recovered show both reducing power and radical scavenging ability, but not antimicrobial activity. The bioaccessibility of the green tea flavonols is reduced in the presence of gelatin used to simulate the presence of protein in the stomach, but it is not clear if this is due to reduced release or sequestration of released compounds by the gelatin.

1. Introduction

The quality and safety of fresh and processed food can be enhanced by the application of edible films and coatings. These edible films can avoid moisture loss, gas exchange, oxidation, photo degradation and control surface microbial spoilage and contamination. Furthermore, safety, nutritional and even sensory properties of edible films and coatings can be improved by the addition of several active ingredients into the polymer matrix (Gómez-Estaca, López De Lacey, Gómez-Guillén, López-Caballero, & Montero, 2009; Gómez-Estaca, López de Lacey, López-Caballero, Gómez-Guillén, & Montero, 2010; Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2009). Green tea extract has been recently used as a safe active ingredient in edible films for the improvement of physical and antimicrobial properties (Hong, Lim, & Song, 2009; Kim et al., 2006).

Green teas are rich in flavan-3-ols (epicatechin, catechin and galloylated derivates), which are responsible for the majority of biological activities. Other polyphenols present in green teas are the flavonols which are mainly glycosylated derivatives of quercetin and kaempferol. Green tea also contains pigments, amino acids, vitamins, carbohydrates, minerals and purine alkaloids (Graham, 1992). The beneficial effects of green tea have been widely described (Graham, 1992) and attributed to many of these compounds, but mainly to phenolic compounds and their antioxidant properties (Bolling, Chen, & Blumberg, 2009; Weisburger & Chung, 2002). When incorporated into edible films, green tea extract can have two functions: active packaging (improving shelf life and quality of food) (Hong et al., 2009) and as a vehicle to deliver compounds which may have potential beneficial effects when the edible film is consumed and releases the polyphenols.

In the literature there are scarcely any studies on the simulated digestibility of edible films and/or on the release of active compounds incorporated into them. However, in order to retain the potential health benefits of green tea components incorporated into edible films, it is essential to know if they are released during digestion. Only the polyphenols released

Abbreviations: EGC, (−)-epigallocatechin; C, (+)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-gallate; EGCG, (−)-epigallocatechin gallate; DGM, dynamic gastric model; HPLC, high performance liquid chromatography; FRAP, ferric reducing ability of plasma; ABTS, 2,2′-azino-*bis*-(3-ethylbenzothiazoline-6-sulfonic acid); VCEAC, vitamin C equivalent antioxidant capacity; T, green tea extract; FT, agar–green tea film; TG, green tea extract + gelatin; FTG, agar–green tea film + gelatin.

☆ This centre has implemented and maintains a Quality Management System which fulfils the requirements of the ISO standard 9001:2000.

* Corresponding author. Tel.: +34 91 5492300; fax: +34 91 5493627.
E-mail address: bgimenez@ictan.csic.es (B. Giménez).

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from solid matrices become bioaccessible and are potentially available for absorption by the gastro-intestinal tract and, therefore, able to exert their beneficial effects in the human body (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). Other factors involved in the bioaccessibility of polyphenols are the transformations (degradation, epimerization, hydrolysis and oxidation) suffered by polyphenols under gastro-intestinal conditions and the interaction between these compounds and food components, which may also modify the biological activity of the phenolic compounds. Interactions between phenolic compounds and some dietary factors, for example proteins and iron, can alter the biological properties and bioaccessibility of polyphenols (Argyri, Proestos, Komaitis, & Kapsokefalou, 2005). Proteins can link to polyphenols and form protein–polyphenol complexes by multiple weak interactions (mainly hydrophobic) or strong interactions (covalent bonds) (Ishii et al., 2008; von Staszewski, Pilosof, & Jagus, 2011). These interactions are affected by the nature of the protein, the temperature of the system and the presence of other components (von Staszewski et al., 2011).

In this study green tea extract and green tea edible films were subjected to simulated human digestion in the upper gastrointestinal (GI) tract (gastric and duodenal digestion). The release of total polyphenols as well as the major flavan-3-ol and flavonols of the green tea was determined in aliquots collected at different times during *in vitro* GI digestion of both tea extract and edible films. Furthermore, the antioxidant and antimicrobial activities were determined in these aliquots and compared with those of the green tea extract. To assess the effect of the well known interactions between protein and polyphenols, gelatin was added to simulate the presence of dietary protein during the digestion.

2. Materials and methods

2.1. Chemical and enzymes

Standard polyphenol compounds: (−)-epigallocatechin (EGC; CAS: 970-74-1), (+)-catechin (CAS:154-23-4), (−)-epicatechin (EC; CAS: 490-46-0), (−)-epicatechin-3-gallate (ECG; CAS: 1257-08-5), (−)-epigallocatechin gallate (EGCG; CAS: 989-51-5), rutin (CAS: 153-18-4), Hyperoside (CAS: 482-36-0), quercetin-3-*O*-glucoside (CAS: 482-35-9), kaempferol-3-*O*-rutinoside (CAS: 17650-84-9), kaempferol-3-*O*-glucoside (CAS: 480-10-4) were purchased from Extrasynthese (Genay, Cedex, France).

In vitro digestion: Egg L-α-phosphatidylcholine (PC, lecithin grade 1, 99% purity) was obtained from Lipid Products (South Nutfield, Surrey, U.K.). Porcine gastric mucosa pepsin (activity of 3300 U/mg of protein calculated by using hemoglobin as substrate), bovine α-chymotrypsin (activity of 40 U/mg of protein using benzoyl-L-tyrosine ethyl ester as substrate), α-amylase (activity 10 U/mg of solid using starch as substrate), porcine trypsin (activity of 13,800 U/mg of protein using benzoyl-L-arginine ethyl ester as substrate), porcine pancreatic lipase (activity of 25,600 U/mg protein), cholesterol, porcine colipase, sodium taurocholate and sodium glycoldeoxycholate were obtained from Sigma (Poole, Dorset, U.K.). Lipase for the gastric phase of digestion was a gastric lipase analogue of fungal origin (F-AP15; activity of ≥150 U/mg) from Amano Enzyme (Nagoya, Japan). Porcine gelatin (60 bloom), 2,4,6-tripyridyl-s-triazine, $FeCl_3$, $FeSO_4$, ABTS radical [2, 2′-azino-*bis*-(3-ethylbenzothiazoline-6-sulfonic acid)], potassium persulphate, and L-ascorbic acid were purchased from Sigma-Aldrich, St. Louis, Mo., USA.

2.2. Extraction of green tea

Chinese green tea known as *Wu Lu Mountain* (*Camellia sinensis* L.) was purchased from a local specialized tea store. The dry green tea was ground into powder using a blender (model 4253–50, Oster, Madrid, Spain). To prepare water extract, the powder (35 g) was mixed with MilliQ water (350 mL) at 80 °C for 30 min, with continuous stirring. The slurry was centrifuged at 12 000 *g* for 10 min at 5 °C. The decanted supernatant was filtered twice through Whatman No. 1. The filtered extract was stored at −20 °C until preparation of the film and analysis.

2.3. Preparation of films

Agar film forming solutions were prepared by dissolving 1.5 g of agar (Gold Agar, Hispanagar, Burgos, Spain) and 1 g of glycerol in 100 ml of water (agar film, F) or a 50/50 v/v mixture of distilled water and green tea extract (agar–green tea film, FT). The mixtures were stirred to obtain a good blend, and the films made by casting 40 ml on 144 cm^2-square plates, drying afterwards at 40 °C in a forced-air oven for 16–18 h to yield a uniform thickness in all cases [200 μm ($p \leq 0.05$)]. Films were conditioned in desiccators for 2 days at 22 °C at 58% relative humidity.

2.4. In vitro digestion studies

Duplicate *in vitro* digestions under gastric and duodenal conditions with and without gelatin (16 mL of porcine gelatin solution at 0.5% per digestion) were carried out on: green tea extract (T), agar–green tea film (FT), green tea extract + gelatin (TG) and agar–green tea film + gelatin (FTG). The agar film without green tea (F) extract was used as a control to check for interference in the analysis of polyphenols by digestion products.

Before performing the simulated human digestion, 20 mL of green tea extract, volume necessary to prepare 1.1 g of film, was mixed with 20 mL of simulated gastric secretion and made up to 240 mL with sterile water. The film (1.1 g) was mixed with the same proportions of sterile water (240 mL) and gastric solution (20 mL) before the *in vitro* digestion studies. Porcine gelatin solution (16 mL) at 0.5% was added to the sterile water in the samples with gelatin, keeping the same proportion previously mentioned. The samples thus dissolved were fed to the dynamic gastric model (DGM), used to provide a realistic and predictive simulation of human gastric processing. The DGM is a computer controlled gastric model which incorporates the chemical, biochemical, physical environment and processes of the human stomach, based on the kinetic data derived from Echo planar-MRI and data on the rates of GI digestion obtained from human studies via naso-gastric and naso-duodenal aspiration and ileostomy patients (Marciani et al., 2007, 2008).

The simulated gastric secretions, bile and pancreatic bile, were prepared as previously reported (Pitino et al., 2010) with some modifications. The DGM works in real time to produce a variable number of samples simulating the digesta that would be emptied from the antrum into the duodenum (Pitino et al., 2010). The digestion conditions of each sample were such as to provide 6 samples over the total gastric digestion time of 24 min, and a gastric emptying rate of approximately 11.3 mL/min. Each gastric sample was weighed, the pH measured and adjusted to 6.8 with NaOH in order to inhibit gastric enzyme activity before performing the determinations.

2.4.1. Simulated gastric secretions

The simulated gastric acid solution contained HCl (0.2 M), NaCl (58 mM), KCl (30 mM), $CaCl_2$ (0.5 mM) and NaH_2PO_4 (0.9 mM). The simulated gastric enzyme solutions were prepared by dissolving porcine gastric mucosa pepsin (8962 U/mL) and gastric lipase (60 U/mL) in the above described salt mixture (no acid). A solution of single shelled lecithin liposomes prepared as described by (Mandalari et al., 2008) was added to the gastric enzyme solution at a final concentration of 0.38 mM.

2.4.2. Simulated bile

Simulated bile contained lecithin (6.5 mM), cholesterol (3 mM, sodium taurocholate (12.5 mM) and sodium glycodeoxycholate (12.5 mM) in a salt solution made of NaCl (146.0 mM), $CaCl_2$ (2.6 mM) and KCl (4.8 mM). The suspension was covered with a blanket of nitrogen,