LWT - Food Science and Technology 53 (2013) 445–451

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

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

Antioxidant properties of green tea extract incorporated to fish gelatin films after simulated gastrointestinal enzymatic digestion

B. Giménez [a,*], S. Moreno [b], M.E. López-Caballero [a], P. Montero [a], M.C. Gómez-Guillén [a]

[a] Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN), CSIC**, C/José Antonio Novais 10, 28040 Madrid, Spain
[b] Fundación Instituto Leloir – Instituto de Investigaciones Bioquímicas Buenos Aires I.I.B.B.A-CONICET, Av. Patricias Argentinas 435, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 12 October 2012
Received in revised form
11 March 2013
Accepted 26 March 2013

Keywords:
Gelatin films
Green tea
Antioxidant
Enzymatic digestion

ABSTRACT

A green tea aqueous extract was prepared and blended at different percentages (2, 4 and 8%) with a commercial fish-skin gelatin in order to provide gelatin films with antioxidant capacity. This green tea extract proved to be an efficient antioxidant at non-cytotoxic concentrations. Gelatin films with green tea extract were subjected to enzymatic digestion with pepsin (gastric digestion) and with pepsin, trypsin and chymotrypsin (gastrointestinal digestion). The gelatin matrix was efficiently hydrolysed during gastrointestinal digestion and protein hydrolysates composed of low molecular weight peptides, regardless the content of green tea extract, were obtained in all the formulations. High percentages of total polyphenols were recovered from the films with green tea extract after gastrointestinal digestion, although a significant degradation of the major catechins of the green tea (EGCG and EGC) was observed. The increase of the content of green tea extract in the film formulation gave an increase in the antioxidant activity released from the film samples after enzymatic digestion. 85–100% of the maximum expected antioxidant activity was recovered after both gastric and gastrointestinal digestion in spite of the degradation observed of EGCG and EGC.

1. Introduction

Green tea (*Camellia sinensis* L.) is a source of polyphenolic compounds having strong antioxidant and antimicrobial activity (Almajano, Carbó, Jiménez, & Gordon, 2008; Chan, Lim, & Chew, 2007). Catechins are the major polyphenols in tea leaves. Besides flavanols, various flavonols and flavones are also present (Chan et al., 2007).

A number of recent studies have dealt with extending the functional properties of biodegradable films by adding different compounds with antioxidant or antimicrobial activities in order to yield a biodegradable active packaging material. Green tea extracts have been already incorporated in food products to extend its shelf life (Martín-Diana, Rico, & Barry-Ryan, 2008), and more recently they have been used as active compound in packaging films (López-de-Dicastillo et al., 2011). Although films generally represent only a minor portion of the food they cover, the study of their digestibility properties may provide useful information for extending potential uses for this type of material in the design of functional foods. In spite of the abundant information on physicochemical and technological features of edible packaging films, research on their digestibility is scarce. Furthermore, most of these studies are focused on the evaluation of changes in the nutritional properties of the biopolymers that constitute the films during and after film preparation (Hernández, Emaldi, & Tovar, 2008; Ou, Kowk, & Kang, 2004); but are not dealing with the residual activity of active compounds incorporated to the edible films after a simulated digestion. When a green tea extract is incorporated in edible packaging films, besides improving shelf life and quality of foods, green tea may have potential health benefits for the consumer when the edible films are consumed and the polyphenols are released. The health benefits of green tea consumption may include improving blood flow, preventing cardiovascular disease or improving resistance to various diseases (Afaq, Adhami, Ahmad, & Mukhtar, 2004).

Fish gelatin films with green tea extract were prepared in this study. Total polyphenol content, antioxidant capacity and cytotoxicity of the green tea extract were determined in order to establish the potential functionality in food packaging applications. The effect of enzymatic digestion on the gelatin matrix as well as on the residual antioxidant activity of tea polyphenols was evaluated.

Abbreviations: EGC, (−)-epigallocatechin; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-gallate; EGCG, (−)-epigallocatechin gallate.

* Corresponding author. Tel.: +34 31 5492300; fax: +34 91 5493627.
E-mail address: bgimenez@ictan.csic.es (B. Giménez).

0023-6438/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lwt.2013.03.020

Furthermore, the recovery of total polyphenols as well as the major catechins after gastrointestinal digestion was also determined.

2. Materials and methods

2.1. Materials

(−)-epigallocatechin (EGC), (+)-catechin, (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin gallate (EGCG), rutin, hyperoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside were purchased from Extrasynthese (Genay, Cedex, France). Type A warm-water fish gelatin was supplied by Rousselot S.A.S. (Puteaux, France). TPTZ (2,4,6-tripyridyl-s-triazine), $FeCl_3$, $FeSO_4 \cdot 7H_2O$, ABTS radical [2, 2′-azino-*bis*-(3-ethylbenzothiazoline-6-sulfonic acid)], Folin–Ciocalteu reagent, gallic acid, sodium carbonate, potassium persulphate, L-ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), porcine gastric mucosa pepsin (3300 U/mg of protein using haemoglobin as substrate), bovine α-chymotrypsin and porcine trypsin (40 U/mg and 13,800 U/mg of protein using benzoyl-L-tyrosine ethyl ester as substrate, respectively) were purchased from Sigma–Aldrich (St. Louis, Mo., USA). The MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) salt and PMS (phenazine methosulfate) were supplied by Promega Biotech Ibérica (Madrid, Spain).

2.2. Preparation and characterization of green tea extract

2.2.1. Preparation of green tea extract

Chinese green tea known as *Lung Ching* (*C. sinensis* L.) was purchased from a specialized tea store (Madrid, Spain). The dry green tea was ground into powder using a blender (Oster, Madrid, Spain). The powder (35 g) was mixed with distilled water (350 mL) and heated at 80 °C for 30 min with continuous stirring. The slurry was centrifuged (12,000 g/10 min, 5 °C). The supernatant was filtered twice (Whatman n° 1) and stored at −20 °C.

2.2.2. Determination of phenols compounds

2.2.2.1. Determination of total phenolic. The total phenolic content in tea extract was determined spectrophotometrically (UV-1601, model CPS-240, Shimadzu, Japan) using Folin–Ciocalteau reagent according to a modified method of Slinkard and Singleton (1977). An aliquot of extract (20 μL) was mixed with 1.58 mL distilled water and oxidized with 100 μL Folin–Ciocalteau reagent. The reaction was alkalinized with 300 μL of 20% sodium carbonate solution and incubated for 2 h at room temperature. The absorbance was measured at 765 nm. Gallic acid (GA) was used as standard and total phenolic content was expressed as mg GA equivalent/mL of tea extract. All determinations were performed at least in triplicate.

2.2.2.2. Quantification by HPLC. Reverse phase high performance liquid chromatography was performed to analyse phenolic compounds in the green tea extract. The separation module consisted of a model SPE-MA10AVP HPLC–UV (Shimadzu, Kyoto, Japan), equipped with a C18 column (25 × 0.78 cm, 5 μm, C18 Tracer Excel 120 ODS-A column, Teknokroma, Spain) and a UV–Vis detector (SPD-10V). The samples were eluted with a gradient system consisting of a solvent A (Milli-Q water) and solvent B (acetonitrile) both containing 1% formic acid at a flow rate of 2.8 mL/min. The temperature of the column was 25 °C and the injection volume was 20 μL. The gradient system started at 95% solvent A and decreased to 75% A over 70 min, followed by a further decrease to 35% solvent A over 10 min. The final conditions were held for 10 min. The peaks of the phenolic compounds were monitored by absorbance at 210 nm and 350 nm. Individual compounds were quantified using a calibration curve of the corresponding standard compound. All analyses were performed in triplicate.

2.2.3. Antioxidant activity

2.2.3.1. Ferric reducing ability of plasma (FRAP). The FRAP assay was carried out according to the method described by Benzie and Strain (1996) with some modifications. The sample (30 μL) was incubated (37 °C) with 90 μL of distilled water and 900 μL of FRAP reagent, containing 10 mM TPTZ and 20 mM $FeCl_3$. Absorbance values were read at 595 nm after 30 min using an UV-1601 spectrophotometer (CPS-240, Shimadzu, Japan). Results were expressed as μmol Fe^{2+} equivalents/mL of sample based on a standard curve of $FeSO_4 \cdot 7H_2O$. All determinations were performed at least in triplicate.

2.2.3.2. ABTS. The ABTS radical scavenging capacity was carried out according to the method of Re et al. (1999). Results were expressed as mg of Vitamin C Equivalent Antioxidant Capacity (VCEAC)/mL of sample based on a standard curve of L-ascorbic acid. All determinations were performed at least in triplicate.

2.2.3.3. DPPH. This assay was carried out as described by Brand-Williams, Cuvelier, and Berset (1995) with some modifications (Fukumoto & Mazza, 2000). The green tea extract was analysed by triplicate, testing at least five concentrations ranging from 2.5 to 20 μg dry matter/mL. The radical scavenging activity was expressed as IC_{50} value, the concentration necessary to quench 50% of initial DPPH radical. Trolox was used as reference compound. The DPPH radical scavenging capacity of Trolox, expressed as IC_{50} value, was determined by testing concentrations ranging from 3 to 50 μg/mL.

2.2.4. Cytotoxic effect of green tea extract

2.2.4.1. Culture of cell lines. The mouse fibroblast cell line 3T3-L1 was grown in DMEM medium (4.5 g/L glucose) supplemented with 10% foetal bovine serum (FBS), at 37 °C under a humidified 5% CO_2 atmosphere. When the cells were approximately 70% confluent, they were split by mild trypsinization and seeded into 24-well plates (1 x 10^4 cells/well). The 24-well plates were incubated at 37 °C/24 h and 5% CO_2. Runs were performed in triplicate with different passage cells.

2.2.4.2. Experimental treatments. After 1 day of incubation, cultures were exposed to increasing concentrations of green tea extract (25–300 μg dry matter/mL) sterilized by filtration and diluted in DMEM medium with 10% FBS. Controls (containing only the culture medium) were included in each plate. The plates were incubated at 37 °C with 5% CO_2 for 24 h.

2.2.4.3. MTS assay. The viability of the 3T3-L1 cells treated with green tea extract for 24 h was determined by the MTS assay, composed of the tetrazolium salt MTS and an electron coupling reagent (PMS). A coloured aqueous soluble formazan product is formed from the tetrazolium compound by mitochondrial activity of viable cells at 37 °C. The amount of formazan produced is directly proportional to the number of living cells in culture. A combined MTS/PMS solution (20:1) was added to the culture medium in a ratio of 1:5 (reagent mixture:medium). After 1 h of incubation, absorbance was measured in a microplate reader at 485 nm (Appliskan, Thermo Scientific, Madrid, Spain).

2.3. Preparation of films

Gelatin control film (0% of green tea extract, C) was prepared by dissolving fish gelatin in distilled water (4 g/100 mL). Gelatin films containing green tea extract were obtained by dissolving gelatin