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Impact of microencapsulation within electrosprayed proteins on the formulation of green tea extract-enriched biscuits

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ABSTRACT

In this work, a green tea extract (GTE) was encapsulated within electrosprayed protein (i.e. gelatin and zein) microparticles, and the protective ability of both systems on the green tea catechins was assessed. The microparticles (with encapsulation efficiencies ~90 g/100 g), proved to be very effective in stabilizing the catechins during a thermal treatment at 180 °C (12 min), preserving 85–90 g/100 g of their initial catechins content, while free GTE lost almost 40 g/100 g of its catechins content. In order to assess the impact of microencapsulation in a real food system, the GTE-loaded electrosprayed microparticles were added to biscuits dough. Results showed that microencapsulation did not significantly protect during biscuit processing and emphasized the need of assessing the behaviour of microencapsulation systems in real food processing conditions. The sensorial analysis of the biscuits indicated that addition of the GTE-loaded microparticles did not impact the acceptability of the biscuits, as perceived by consumers.

1. Introduction

Green tea catechins are a group of polyphenolic antioxidants which have received increasing research attention in recent years because of their reported health promoting properties, such as anti-carcinogenic (Larsen & Dashwood, 2010) and antimicrobial effects (Steinmann, Buer, Pietschmann, & Steinmann, 2013). Among them, (−)-epigallocatechin gallate (EGCG) is the most abundant and bioactive flavonoid in green tea (Barras et al., 2009). Together with (−)-epicatechin gallate (ECG) it is accepted as an indicator of the quality of green tea extracts (GTE) (Sharma & Zhou, 2011). These products are being increasingly added to a variety of foods in order to improve their nutritional value (Ananingsih, Gao, & Zhou, 2013) and make them more appealing to consumers (Yilmaz, 2006), whose interest towards functional foods is increasing (Siró, Kápolna, Kápolna, & Lugasi, 2008).

Despite the promising benefits of supplementing food products with GTE, there are a number of technological concerns due to the poor stability of tea catechins, which are thermosensitive and highly unstable in alkaline conditions, especially EGCG and ECG (Su, Leung, Huang, & Chen, 2003), so their bioactivity could be compromised upon food processing and storage. Moreover, GTE has been reported to negatively impact aroma, flavour and overall acceptability of bread and, thus, the quality of the final food products (Bajerska, Mildner-Szkudlarz, Jeszka, & Szwengiel, 2010). Being one of the most popular bakery products consumed by nearly all levels of society and all age groups (Tarancón, Fiszman, Salvador, & Tárrega, 2013), biscuits are regarded as good candidates to be fortified with functional ingredients (Choudhury, Badwaik, Borah, Sit, & Deka, 2015). They are convenient snacks with a long shelf life and appealing sensory attributes (Fradinho, Nunes, & Raymundo, 2015). In fact, the supplementation of biscuits with GTE (Mildner-Szkudlarz, Zawirska-Wojtasiak, Obuchowski, & Gośliński, 2009; Sharma & Zhou, 2011) or grounded tea leaves (Gramza-Michałowska et al., 2016) has already been attempted with different purposes. However, biscuit doughs generally have high pH values, and high temperatures are used to bake them, threatening the stability of tea catequins if supplemented with GTE (Sharma & Zhou, 2011).

Microencapsulation, or entrapment of an ingredient within a

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protective micron-sized matrix, is a plausible approach to protect sensitive bioactive molecules against degradation (Ye, Cui, Taneja, Zhu, & Singh, 2009). Electrospraying has recently emerged as an alternative to traditional microencapsulation techniques in the food industry such as spray-drying (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007), as it can be performed under mild temperatures (López-Rubio & Lagaron, 2012), being specially adequate for the encapsulation of thermosensitive functional ingredients (Gómez-Mascaraque & López-Rubio, 2016). This technology, based on electrohydrodynamic processing, consists of subjecting a polymer solution to a high voltage electric field in such a way that a charged polymer jet is ejected towards a grounded collector, generating dry polymeric particles as the jet breaks down into fine droplets during its flight and the solvent evaporates (Bhardwaj & Kundu, 2010). For food applications, solutions or suspensions of edible biopolymers such as proteins or polysaccharides containing the ingredient to be encapsulated can be electrosprayed using food-grade solvents (Gómez-Mascaraque, Ambrosio-Martín, Fabra, Pérez-Masiá, Lagaron, & López-Rubio, 2016). Furthermore, these food macromolecules are known to interact with polyphenols (Jakobek, 2015; Le Bourvellec & Renard, 2012), which may contribute to the stabilization of these bioactive compounds when encapsulated within them (Gómez-Mascaraque, Lagarón, & López-Rubio, 2015).

In previous work, we demonstrated the potential of electrosprayed gelatin submicroparticles to protect EGCG against degradation in alkaline conditions (Gómez-Mascaraque et al., 2015). Zein fibers produced through electrohydrodynamic processing have also been reported to be effective in stabilizing EGCG in aqueous environment (Li, Lim, & Kakuda, 2009). The aim of the present study was to evaluate GTE-containing microparticles produced through electrospraying using both protein matrices when applied to a real food product. Specifically, they were added to biscuit dough. The protection exerted by the encapsulation matrices on the catechins upon biscuit preparation was evaluated by comparing the loss of these compounds and their antioxidant activity in comparison with the direct addition of the non-encapsulated GTE to the biscuit dough. A sensorial analysis was also conducted to assess whether the incorporation of the GTE and/or the microparticles to the biscuit formulation had an impact on the consumers' acceptability.

2. Materials and methods

2.1. Materials and ingredients

Type A gelatin from porcine skin (Gel), with reported gel strength of 175 g Bloom, and zein prolamine from corn, grade Z3625, were both purchased from Sigma-Aldrich and used as received without further purification. A green tea extract (GTE) with high oxygen radical absorbance capacity (ORAC) was kindly donated by Naturex (Avignon, France). Potassium persulfate ($K_2O_8S_2$), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium bromide FTIR grade (KBr), phosphoric acid (H_3PO_4), pyrogallol and ascorbic acid were obtained from Sigma-Aldrich. Acetic acid (96 mL/100 mL) was purchased from Scharlab, ethanol (96 mL/100 mL) from Panreac and acetonitrile from Biosolve Chimie (Dieuze, France). (+)-catechin (C), (−)-gallocatechin (GC), EGCG, ECG, (−)-epigallocatechin (EGC), (−)-epicatechin (EC) and (−)-gallocatechin gallate (GCG) standards were purchased from Sigma Aldrich.

The ingredients used to prepare the biscuits were 350 g soft wheat flour (Belenguer, S.A. Valencia) with 11 g/100 g protein, 0.6 g/100 g ash and alveograph parameters of P/L = 0.27 and W = 134, as provided by the supplier, where P is the maximum pressure required, L the extensibility and W the baking strength of the dough; 112.52 g vegetable margarine (Unilever, Spain), 103.07 g sugar (Azucarera Ebro, Madrid, Spain), 37.62 g reconstituted skimmed milk (from 6.12 g skimmed milk powder, Central Lechera Asturiana, Spain), 3.67 g salt, 1.22 g sodium bicarbonate (A. Martínez, Cheste, Spain) and 0.70 g ammonium hydrogen carbonate (Panreac Quimica, Barcelona, Spain).

2.2. Catechins profile of the GTE

High-performance liquid (HPLC) was used to identify the catechins profile of the GTE. The LC system used was an Agilent 1200 series (Agilent Technologies, Santa Clara, USA) equipped with a binary pump and a diode-array-detection (DAD) system. Separation of the different catechins (i.e. C, GC, EGCG, ECG, EGC, EC and GCG) was performed using a Zorbax SBC_8 (150 × 4.6 mm, 3.5 μm of particle size) LC-column. The flow rate was set to 0.9 mL/min and the oven temperature was 30 °C. Eluent A was acetonitrile and eluent B water slightly acidified with 0.1 g/100 g H_3PO_4. The elution gradient started with 100 mL/100 mL of eluent B, decreasing to 90 mL/100 mL B in 20 min and to 85 mL/100 mL in 60 min. The injection volume was 2 μL. Acquisition was done using the ChemStation software (Agilent Technologies). The detection wavelength was set at 280 nm. The catechins were identified based on their retention times, as compared with their standards.

2.3. Microencapsulation of the GTE

The GTE was microencapsulated through electrospraying within two different protein matrices: gelatin and zein. For this purpose, solutions of the proteins in adequate solvents were first prepared. Gelatin aqueous solutions (8 g/100 mL) were prepared in diluted acetic acid (20 mL/100 mL) as described in Gómez-Mascaraque et al. (2015), and cooled down to room temperature before addition of the GTE. On the other hand, zein (12 g/100 mL) was dissolved in ethanol (85 mL/100 mL) at room temperature (concentrations selected from preliminary tests in order to obtain electrosprayed particles as free of fibres as possible). The GTE was then added to the protein solutions under magnetic stirring at a concentration of 20 g/100 g of the total solids content in both cases.

The GTE-protein solutions were processed using a homemade electrospraying apparatus, equipped with a variable high-voltage 0–30 kV power supply. The solutions were introduced in a 5 mL plastic syringe and pumped at a constant flow-rate through a stainless-steel needle (0.9 mm inner diameter). The needle was connected through a PTFE wire to the syringe, which was placed on a digitally controlled syringe pump. Processed samples were collected on a grounded stainless-steel plate, placed facing the syringe in a horizontal configuration, at a distance of 10 cm, as illustrated in Fig. 1. The voltage applied to the needle and the pumping flow-rate, respectively, were 15 kV and 0.2 mL/h for the gelatin solutions (Gómez-Mascaraque et al., 2015), and 13 kV and 0.15 mL/h for the zein solutions (conditions selected from preliminary tests).

2.4. Characterization of the microparticles

Scanning electron microscopy (SEM) was conducted on a Hitachi S-4800 microscope (Hitachi High-Technologies, Tokyo, Japan) at an accelerating voltage of 10 kV and a working distance of 8–9 mm. Samples were sputter-coated with a gold–palladium mixture under vacuum prior to examination. Particle diameters were measured from the SEM micrographs in their original magnification using the ImageJ software. Size distributions were obtained from a minimum of 200 measurements.

The samples were also subjected to Fourier transform infrared