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Antimicrobial and magnetically removable tannic acid nanocarrier: A processing aid for *Listeria monocytogenes* treatment for food industry applications

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ABSTRACT

An innovative core-shell nanocarrier, combining the magnetism of surface active maghemite nanoparticles (SAMNs, the core) and tannic acid (TA, the shell) was self-assembled by simple incubation in water. Due to the drastic reorganization of SAMN surface, the prepared magnetic nanocarrier (SAMN@TA) resulted as one of the most robust nanomaterial bearing TA to date. Nevertheless, the ferric tannates network, constituting the SAMN@TA shell, and the free tannic acid display comparable chemical behavior. The antimicrobial properties of SAMN@TA were tested on *Listeria monocytogenes* in comparison with free TA, showing similar bacteriostatic effects at relatively low concentrations. Besides the preservation of the TA inhibitory activity toward *L. monocytogenes*, the possibility of being magnetically removed leaving no residues into the matrix makes this nanocarrier an innovative processing aid for surface treatments. Thus, SAMN@TA can be used as an effective, low-cost and environmentally friendly antimicrobial nanomaterial for the food industry applications.

1. Introduction

The control of foodborne pathogens represents a crucial task as a mean to reduce significantly the possibility of disease outbreaks (Kiran-kumar, Badarinath, & Halami, 2008). In an attempt to prevent this problem, food industries normally use synthetic preservatives and antibiotics in the food production processes for eliminating microorganisms. However, the combination of excessive and inappropriate use of chemical substances led to the emergence of drug resistance in bacteria, increasing the difficulty of controlling the proliferation of foodborne diseases.

The interest in natural alternatives to antibiotics and classical preservatives is rapidly increasing for reducing chemicals and avoiding bacterial resistance, and may represent a new strategy against microbial infections (David, Steenson, & Davidson, 2013). Among natural substances, phenolic compounds have been shown to exert antioxidant, anti-inflammatory, anti-cancer and antibacterial activity. Indeed, phenolic compounds are a class of biomolecules known for their antimicrobial action (Coppo & Marchese, 2014; Gyawali & Ibrahim, 2014; Kim, Kang, & Kim, 2011; Lagha, Dudonné, Desjardins, & Grenier, 2015), and were proposed as natural food preservatives against foodborne bacteria (Coppo & Marchese, 2014).

Phenolic compounds are widely distributed in natural products, such as fruits, vegetables and seeds, and constitute one of the largest and most ubiquitous class of secondary metabolites in the plant kingdom. They comprise, as common motif, molecular structures with at least one aromatic ring, substituted with one or more hydroxyl moieties (Fig. 1, panel A). Most phenols of natural origin have at least two hydroxyls in their structure and are characterized by a wide structural diversity.

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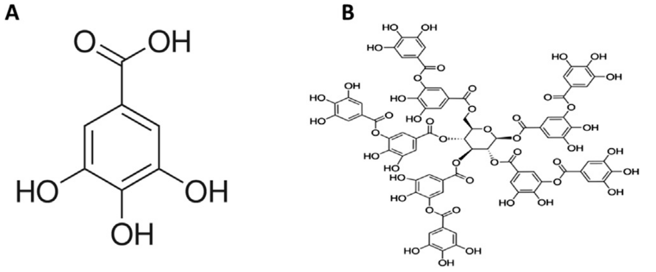

Fig. 1. Structure of a simple phenolic compound and of tannic acid. A) Molecular structure of gallic acid B) Molecular structure of tannic acid.

Among phenolic compounds, tannic acid (TA), is a widely spread polyphenol, characterized by an effective inhibition of bacterial growth, see panel B of Fig. 1 (Coppo & Marchese, 2014). Molecularly, TA (IUPAC name: 2,3-dihydroxy-5-({[(2R,3R,4S,5R,6R)-3,4,5,6-tetrakis({3,4-dihydroxy-5-[(3,4,5-trihydroxyphenyl)-carbonyloxy]-phenyl}carbonyloxy)oxan-2-yl]methoxy}carbonyl)phenyl-3,4,5-trihydroxybenzoate) affects several metabolic pathways in bacteria cells, where it can reduce iron intake, due to its chelating properties toward transition metals, leading to a modulation of cellular oxidative stress (Lim, Penesyan, Hassan, Loper, & Paulsen, 2013). Bacterial swimming motility and biofilm formation are also affected by TA, possibly due to iron ions depletion or to the direct binding of TA to cell surface structures, such as lipo-polysaccarides and flagellin (Lim et al., 2013; O'May & Tufenkji, 2011). These effects are of greater concerns for the food industry, where biofilm formation is a matter of concern, especially for foodborne pathogen, such as *Listeria monocytogenes*.

L. monocytogenes can cause listeriosis, which is a severe disease with high hospitalization and a case-fatality rate up to 30% (Buchanan, Gorris, Hayman, Jackson, and Whiting, 2017; Lomonaco, Nucera, & Filipello, 2015; Mead et al., 1999; Muñoz et al., 2012). Both outbreaks and sporadic cases are associated with contamination of various types of food, such as milk, soft cheese, meat, vegetables and seafood products. The ubiquitous nature of this foodborne pathogen and its growth ability at low temperatures concur to its survival and maintenance in the food industry plants. Indeed *L. monocytogenes* can persist for long time in the form of biofilm on the surfaces of the processing plant, and even in the refrigerated food final products (Simões, Simões, & Vieira, 2010).

The use of natural compounds could be a new eco-friendly system to control *L. monocytogenes* contamination of food and biofilm formation. The strong activity of tannin rich fraction of pomegranate against *L. monocytogenes* cell membrane, was documented (Li et al., 2014). Accordingly, Cetin-Karaca and Newman (2015) described the antimicrobial activity of TA on *L. monocytogenes*, showing a minimal inhibitory concentration (MIC) below 20 μg/mL, after 60 h of incubation. Concerning its real applicability in food industry, tannic acid can not be considered a processing aid as it can be hardly removed from food matrixes once added.

In the last years, nanotechnology emerged as sophisticated platform for responding to the need of novel strategies at molecular level (Bohara & Pawar, 2015), and represents a new frontier in food industry, finding potential applications in every segment of the production process. Nanomaterials can be employed as supports for the immobilization of substances of interest enhancing their bioavailability, creating magnetically drivable vehicles (Magro, Campos et al., 2014) and providing targeting functionalities, which can improve delivered drug efficacy. Furthermore, nanomaterials can provide competitive methods for the identification, capture and inhibition of bacteria (Inbaraj & Chen, 2015).

Recently, we developed a new type of magnetic nanoparticles, consisting of a nanostructured superparamagnetic iron oxide constituted of stoichiometric maghemite (γ-Fe_2O_3), with a size around 10 nm, and presenting peculiar surface chemical behavior (Magro, Sinigaglia et al., 2012). These nanoparticles were named "surface active maghemite nanoparticles" (SAMNs). SAMNs show excellent water stability, as colloidal suspensions, without any organic or inorganic surface coating, and a high average magnetic moment, allowing an easy magnetic driving. Due to SAMN peculiar properties, they were already used for immobilizing different biomolecules (Magro, Faralli et al., 2012; Magro, Baratella et al., 2015) and proposed for the development of biosensors (Baratella et al., 2013; Bonaiuto et al., 2016; Magro et al., 2013), and for biomedical applications (Cmiel et al., 2016; Skopalik et al., 2014; Venerando et al., 2013), biotechnology (Magro, Faralli et al., 2012) and food industry (Magro, Baratella et al., 2014; Magro, Esteves et al., 2016; Miotto et al., 2016). In particular, we prepared and characterized a novel hybrid nanomaterial by coating SAMNs with TA (SAMN@TA). SAMN@TA complex resulted extremely stable and was structurally and electrochemically characterized and successfully applied for the development of an electrochemical sensor (Magro, Fasolato et al., 2016). In the present report, the antimicrobial properties of the nanostructured SAMN@TA hybrid complex were tested against *Listeria monocytogenes*, and proposed as processing aid for surface treatment, without leaving residues in the matrix.

2. Material and methods

Chemicals were purchased at the highest commercially available purity and were used without further treatment. Iron(III) chloride hexahydrate (97%), sodium borohydride (NaBH4), tannic acid ((IUPAC name: 2,3-dihydroxy-5-({[(2R,3R,4S,5R,6R)-3,4,5,6-tetrakis({3,4-dihydroxy-5-[(3,4,5-trihydroxyphenyl)-carbonyloxy]-phenyl}carbonyloxy)oxan-2-yl]methoxy}carbonyl)phenyl-3,4,5-trihydroxybenzoate, cat. 40304, 1701.23 $g mol^{-1}$ molecular weight), and ammonia solution (35% in water) were obtained from Aldrich (Sigma–Aldrich, Italy).

2.1. Synthesis of nanoparticles

A typical nanoparticles were synthesized as previously described (Magro, Valle, Russo, Nodari, & Vianello, 2012) and can be summarized as follows: $FeCl_3 \cdot 6H_2O$ (10.0 g, 37 mmol) was dissolved in Milli-Q grade water (800 mL) under vigorous stirring at room temperature. $NaBH_4$ solution (2.0 g, 53 mmol) in ammonia (3.5%, 100 mL, 4.86 mol/mol Fe) was then quickly added to the mixture. After the reduction reaction occurrence, the temperature was increased up to 100 °C and kept constant for 2 h, under stirring. Then, the system was cooled at room temperature and aged in water for 12 h. The magnetic product was separated by imposition of an external magnet for 60 min, and washed three times with water. The resulting material can be transformed into a red brown powder (final synthesis product) by curing at 400 °C for 2 h, leading to individual nanoparticles. The resulting nanopowder showed a high magnetic response upon exposure to a magnetic field. The final Fe_2O_3 mass was 2.0 g (12.5 mmol) and a yield of 68% was calculated. The obtained naked iron oxide nanomaterial leads to a stable colloidal suspension in water upon sonication (Bransonic, model 221, 48 kHz, 50 W).

2.2. Magnetic separation

A series of Nd–Fe–B magnets (N35, 263-287 kJ/m^3 BH, 1170-1210 mT flux density by Powermagnet, Germany) were used for

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