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Migration evaluation of silver nanoparticles from antimicrobial edible coating to sausages

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ABSTRACT

Use of silver nanoparticles as an antimicrobial compound directly on foodstuff has been attracted attention due to concerns on the total residual silver that remains on the food after preparation. In this work, silver nanoparticles (AgNPs) were obtained by a green route and applied as edible coating to chicken sausages. Antimicrobial activity of the AgNPs were able to inhibit lactic acid bacteria for 30 days, demonstrating that the increase in the shelf life of the sausages was statistically significant ($P < 0.05$). The presence of AgNPs also affected the texture of the sausages probably due to the interaction between silver and phosphorous and sulphur from proteins. After 30 days, lipid oxidation was found to be higher in treated sausages than in control samples. Sausages were prepared simulating home cooking and the concentration of silver after each step was determined, showing that a simple washing and cooking procedure was able to remove most of the silver from sausages. Total silver concentration on the sausages after that was 5.3 $ng_{AgNPs}/g_{sausage}$. Also, no appreciable migration of silver nanoparticles from sausages surface to its interior was detected.

1. Introduction

Application of edible coatings to meat, poultry, fish, fruits and vegetables has attracted increasing interest since an array of additives can be incorporated to edible coatings formulation providing new functionalities such as antimicrobial and antifungal activity (Campos, Gerschenson, & Flores, 2011; Du, Avena-bustillos, Hua, & Mchugh, 2011). It was demonstrated that food deterioration caused by pathogens and spoilage bacteria can be significantly reduced in meat products by antimicrobial-loaded edible coatings (Alvarez, Ponce, & Moreira, 2013; Brasil, Gomes, Puerta-gomez, Castell-perez, & Moreira, 2012; Cagri, Ustunol, & Ryser, 2004; Fernández-Pan, Carrión-Granda, & Maté, 2014). This is important in the case of meat sausages because microorganism action in anaerobic conditions lead to the formation of surface slime thus decreasing sensory characteristics (De Palo, Maggiolino, Centoducati, & Tateo, 2013).

Silver ions slowly released from silver nanoparticles (AgNPs) present broad antimicrobial spectrum against Gram-negative and Gram-positive bacteria, fungi, protozoa and certain viruses (Kumar & Münstedt, 2005). However, there is still great concerns on silver ingestion limits. Silver presents the lowest toxicity among metals to animal cells, being toxic in concentrations higher than 10 mg L^{-1} (Leite, 2003; Levin et al., 2009). According to the study developed by Kittler, Greulich, Diendorf, Köller, and Epple (2010), silver nanoparticles slowly dissolve into ions in a time scale of several days. Authors evaluated ions release during 125 days at 5, 25 and 37 °C and concluded that the biological action of freshly prepared and aged nanoparticles are strongly different due to the different amount of released ions. Hadrup and Lam (2014) reviewed the oral toxicity of silver ions, silver nanoparticles and colloidal silver. Silver was detected in body tissues such as the skin epidermis, the glomeruli and the intestines after exposure to both ionic and nanoparticulated silver suspensions. In 2010, US EPA (EPA, 2010)

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report stressed that the toxicity potential of nanosized silver to humans depended on the level of exposure and the association with other nano-Ag containing products (Kim et al., 2015).

AgNPs have been already added to edible coating formulations to inhibit microbial growth in shitake mushrooms (Jiang, Feng, & Wang, 2013), minimally processed carrots (Costa, Conte, Buonocore, Lavorgna, & Nobile, 2012) and asparagus (An, Zhang, Wang, & Tang, 2008). Unfortunately, little attention was given to the amount of silver actually ingested or if it is below human toxicity levels. Interaction between AgNPs and the food matrix is also worth investigating because silver may migrate to foodstuff affecting overall properties.

The objective of this work was to investigate silver migration from an edible coating applied to sausages to the food matrix. Also, the influence of the silver nanoparticles on bacterial growth, texture profile and lipid oxidation were determined.

2. Material and methods

2.1. Material

Chicken sausages were acquired from the local market from Campo Mourão, PR, Brazil (all sausages used were from the same 2 kg package). Soluble starch (Merk, Germany), anhydrous D-glucose (Isofar, Brazil) and silver nitrate (Proquímios, Brazil) were used in the silver nanoparticles synthesis. Tryptic soy broth (Biomark, Brazil), Muller-Hinton broth (Biomark, Brazil), MRS agar (Biomak, Brazil) and saline solution were used in the antimicrobial analyses. Malonaldehyde bis (dimethyl acetal) (1,1,3,3-tetramethoxypropane, TMP), 2-thiobarbituric acid, propyl gallate (Sigma Aldrich, Germany), trichloroacetic acid and EDTA (Vetec, Brazil) were used in Thiobarbituric acid-reactive substances (TBARS) assay.

2.2. Silver nanoparticles synthesis

AgNPs were synthesized by the method previously described by Ghaseminezhad, Hamedi, and Abbas (2012) using D-glucose and starch as reducing agent and stabilizer, respectively. Initially, silver nitrate aqueous solution (2 mL, 25 mM) was mixed with starch solution (50 mL, 1%w/w) and aqueous D-glucose solution (4 mL, 25 mM) was added. Finally, the resultant solution was autoclaved (Prismatec equipment, Brazil, 121 °C and 15 psi) for 15 min forming the silver colloidal nanoparticles solution.

2.3. AGNPs characterization

Size distribution, polydispersion index (PDI) and z-average size (Dz) of the synthesized AgNPs were determined by Dynamic Light Scattering (DLS, Malvern Nanosizer, United Kingdom). Morphological analysis of AgNPs was performed by Scanning Electron Microscopy (SEM, Shimadzu SSX 550 Superscan, Japan).

2.4. Sausages coating

AgNPs colloidal solution was diluted to 37.50 μg mL^{-1} since this was the minimum inhibitory concentration (MIC) determined by Pizzoli et al. (2016) against *Staphylococcus aureus* (ATCC 6538). They synthesized AgNPs by the same method used in the present work and determined the MIC of AgNPs against *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Since *Staphylococcus aureus* was the most resistant microorganism, its MIC was chosen as reference concentration. All materials used in the procedure were previously sterilized in an autoclave.

Sausages were immersed during 1 min in the AgNPs solution (37.50 μg mL^{-1}) and, after that, they were kept over a grid to remove the excess of solution. For each storage time interval (0, 15 and 30 days), 3 sausages (approximately 135 g aseptically weighed) were covered with the AgNPs solution and vacuum packaged. For the control samples the same manipulation was applied except by the immersion step. Samples were stored at 10 ± 2 °C. All treatments were carried out in quadruplicate.

2.5. Microbial analysis

The presence of lactic acid bacteria in the sausages samples was determined at different time intervals: 0 (just after AgNPs treatment and packaging), 15 and 30 days. Sausages samples were aseptically weighed (25 g), transferred to sterile plastic pouches and homogenized with sterile saline solution (225 mL) during 1 min in a stomacher (ITR, MR1204, Brazil). Appropriate dilutions of the sample homogenates were prepared in sterile peptone water (0.1%) and inoculated in triplicate in MRS growth media plates (lactic acid bacteria selective agar). The inoculated culture plates were incubated in a bacterial culture incubator (Ethik, Brazil) under aerobic conditions at 37 °C for 48 h and finally the plate counts were determined.

2.6. AGNPs concentration on sausages

Metallic silver concentration was evaluated in sausages during the storage period (15 and 30 days) and home cooking of the sausages was simulated using the following steps. First, sausages from one vacuum package were washed with distilled water (1 L). After that, they were cooked with boiling distilled water (1 L) during 5 min. Finally, the cooked sausages were crushed with distilled water (1 L) in a domestic blender. The crushed sausages sample was centrifuged (Mini Spin Plus Eppendorf centrifuge, Germany, 30 min at 14,500 rpm) and the supernatant was collected. The procedure was adopted in duplicate for all storage time intervals and the samples (washing water, cooking water and crushed sausages) were submitted to Inductive Coupled Plasma with Mass Spectroscopy (ICP-MS, Perkin Elmer, NexIon 300 D, Shelton, USA) analysis. Final results were expressed as metallic silver concentration (μg^{0}_{Ag} mL^{-1}).

2.7. Texture Profile Analysis

Texture Profile Analysis (TPA) was performed with a TA-XT Express Enhanced (Stable Micro Systems, United Kingdom) texture analyzer and the P/36R cylindrical probe was used in the compression tests. Six pieces from each experimental condition (2.0 cm diameter, 2.0 cm height) were compressed twice until 50% of the original sample height. A cross-head speed of 1 mm s^{-1} was applied. The following TPA parameters were computed: hardness, adhesiveness, cohesiveness, springiness, gumminess and chewiness.

2.8. Assessment of lipid oxidation by TBARS assay

Thiobarbituric acid-reactive substances (TBARS) assay was carried out according to the procedure described by Sallam, Ishioroshi, and Samejima (2004) with minor adaptations. Sausage samples (5 g) were mixed with trichloroacetic acid solution (25 mL, 7.5%w/v TCA, 0.1%w/v propyl gallate and 0.1% EDTA) and homogenized in a blender for 30 s. After filtration, 5 mL of the filtrate were added to the TBA solution (5 mL, 0.02 mol L^{-1}) in a test tube. Test tubes were incubated in a boiling water bath during 40 min then the absorbance was measured at 538 nm (UV–Vis spectrophotometer, Ocean Optics, USB650UV, USA). TBA value was expressed as