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Preparation and antimicrobial characterization of silver-containing packaging materials for meat

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ABSTRACT

In food technology, antimicrobial packaging materials could inhibit or limit the growth of spoilage bacteria and thus improve the shelf life of packaged products. The present study provides new insights into the preparation and antimicrobial characterization of silver-containing packaging materials and their efficacy against typical meat spoilage bacteria. Antimicrobial efficacy of packaging films produced by coextrusion or liquid flame spray process was determined by bioluminescence imaging and conventional antimicrobial assay. Fresh pork sirloin was packaged in selected films and composition of meat microbiota was analyzed by 16S rRNA amplicon sequencing. Shelf life of meat was not affected by any of the silver-containing packaging films, even though meat microbiota mostly consisted of bacteria that were inhibited or retarded *in vitro* by nanoscale silver coating. This may be due to different release dynamics of silver ions on meat surfaces compared to the circumstances in the antimicrobial assay or interactions between silver and amino acids.

1. Introduction

Raw red meat has a high water activity and plenty of nutrients that enable bacterial growth. Thus, meat rapidly loses its quality even at chill temperatures because of microbial activity and subsequent sensory changes (Sun & Holley, 2012). Carbon dioxide tolerant psychrotropic lactic acid bacteria (LAB) are typically associated with spoilage of vacuum or modified atmosphere (MA) packaged meat. *Carnobacterium*,*Lactobacillus* and *Leuconostoc* spp. are most commonly detected in spoiled, cold stored meat products packaged under MA (Borch, Kant-Muermans, & Blixt, 1996; Susiluoto, Korkeala, & Björkroth, 2003). Since these bacteria cannot be completely eradicated from raw meat even if high processing hygiene is maintained, new packaging technologies are needed for improving shelf life and quality of the packaged product.

Meat spoilage occurs primarily on the surface of meat. To limit the growth of spoilage microbes, antimicrobial substances have been tested in direct contact with food products by dipping or spraying, even though this kind of approach has relatively short-time effects on microbial growth due to neutralization, diffusion or inactivation of active ingredients (Appendini & Hotchkiss, 2002; Quintavalla & Vicini, 2002). A more durable solution could be the incorporation of antimicrobial agents as a part of the packaging material. Controlled and extended release of antimicrobial agents could inhibit or retard bacterial growth throughout storage, thus leading into benefits in the whole supply chain (Appendini & Hotchkiss, 2002; Han, 2000; Quintavalla & Vicini, 2002).

Silver has been known since historical times for its antimicrobial activity and several mechanisms of activity against bacteria have been proposed (Lalueza, Monzón, Arruebo, & Santamaría, 2011; Silvestry-Rodriguez, Sicairos-Ruelas, Gerba, & Bright, 2007). These include extracellular binding or precipitation of silver to cell walls, active transport of silver into cells *via* transport systems of essential metals, and binding of silver to DNA or electron donor groups. Silver can also bind to sulfhydryl groups (—SH) of proteins, which causes protein inactivation and inhibition of metabolic processes (Silvestry-Rodriguez et al., 2007). Interaction of silver with ribosomes inhibits enzyme expression (Llorens, Lloret, Picouet, Trbojevich, & Fernandez, 2012). Silver can be introduced in different forms such as ions, complexes, salts and in metallic form. Activity of silver ions is dependent on the anions and

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biological molecules of the environment and is also affected by redox processes that depend on light, temperature and oxygen level. In case of nanoparticles, factors such as size, surface area, surface charge and geometry of the particles affect their activity (Lalueza et al., 2011). Silver-exchanged zeolites consist of aluminosilicates where complexed alkaline or earth alkaline metals have been partially replaced with silver ions (Fernández, Soriano, Hernández-Muñoz, & Gavara, 2010).

Although various techniques have been used in preparing silver-containing polymeric materials (Muñoz-Bonilla & Fernández-García, 2012) and antimicrobial effects have been shown *in vitro* in several studies (Boschetto, Lerin, Cansian, Pergher, & Di Luccio, 2012; Dogan, Koral, & Inan, 2009; Fernández et al., 2010; Lalueza et al., 2011; Pehlivan, Balköse, Ülkü, & Tihminlioğlu, 2005), fewer studies have been published about the effects of silver in storage of real food, such as meat (Lee, Lee, Jones, Sharek, & Pascall, 2011), fruit or vegetables (Costa, Conte, Buonocore, & Del Nobile, 2011; Martínez-Abad, Lagarón, & Ocio, 2014) or cheese (Gammariello, Conte, Buonocore, & Del Nobile, 2011; Incoronato, Conte, Buonocore, & Del Nobile, 2011). The aim of this study was to prepare silver-containing packaging materials and evaluate their antimicrobial effect against several bacterial strains associated with meat spoilage. Potential packaging films were selected by initial *in vitro* tests against genetically modified luminescent bacteria and conventional antimicrobial assay against various spoilage bacteria. Selected films were used in packaging of pork sirloin to evaluate their effect on pork shelf life. Characterization of meat spoilage under different packaging conditions was performed by 16S rRNA amplicon sequencing.

2. Materials and methods

2.1. Preparation of packaging materials

2.1.1. Coextrusion

Two silver-containing low density polyethylene (LDPE) masterbatches were used in the trials. A commercial masterbatch contained silver-zinc zeolite in LDPE matrix (Irgaguard® B 5120, BASF, Ludwigshafen, Germany). The other masterbatch was prepared by melt mixing of 4 kg LDPE (CA7230, Borealis, Wien, Austria) and 200 g silver substituted titanium dioxide (SG TP8, Silvergreen Oy Ltd., Helsinki, Finland) in Brabender DSE25 twin screw extruder (Duisburg, Germany) at 200 °C, screw speed 89 rpm, torque 93 Nm and mass pressure 6.0 bar.

Coextruded packaging films were produced in a continuous roll process at Tampere University of Technology (TUT, Finland) on the Paper Converting and Packaging Technology pilot line. Food-grade paperboard (Stora Enso Oyj, Imatra, Finland) was used as a substrate for coating. Silver masterbatches were dry blended with CA7230 to obtain concentrations presented in Table 1. The resulting films consisted of a thin top layer of silver-containing polymer blend and bottom layer of pure CA7230. Total coating weight of approximately 20 g/m^2 was achieved by adjusting the screw speed or line speed. Pure LDPE film (control) was analogously produced. Adhesion between the substrate and the film was adjusted to be very low to enable substrate removal without damaging the produced film.

In this paper, coextruded packaging films are referred to according to their active ingredient (Irgaguard or SG TP8) and its concentration, *e.g.*, Irgaguard 2%. Silver-free coextruded film is referred to as control.

2.1.2. Liquid flame spray

Liquid flame spray (LFS) technology was used for coating LDPE films with nano-scale metallic silver particles. Principles of the process have been described earlier (Aromaa, Keskinen, & Mäkelä, 2007; Aromaa et al., 2012; Teisala et al., 2010; Tikkanen et al., 1997). The laboratory scale conveyor line described by Aromaa et al. (2012) was used in the experiments. Silver nitrate (Sigma–Aldrich, Germany) in ion exchanged H_2O was used as a precursor and coextruded control film sheets (27.5 × 19.5 cm) as substrate for nanoparticle deposition. Size of nanoparticles was adjusted by using either 500 mg/mL (big particle size) or 125 mg/mL (small particle size) precursor concentration. Each substrate sheet was coated 1, 2 or 4 times. Other parameters remained constant: precursor feed rate 2 mL/min, burner distance 20 cm, line speed 50 m/min and gas flow rate 40/20 lpm (H_2/O_2).

In this paper, packaging films prepared by LFS are referred to as B1–B4 (big particle size, coated 1–4 times) or S1–S4 (small particle size, coated 1–4 times). Control film is the same as for coextruded films.

Silver nanoparticles produced by LFS were studied with transmission electron microscopy (TEM). During LFS, nanoparticle deposit was collected on a TEM grid (S160-3, Agar Scientific) by a sampling device (alumina stick and sample holder). The grid was centered on the device and wiped perpendicularly through the flame. Separate grids were produced for big and small particle size. The JEOL JEM-2010 instrument operating at 200 kV acceleration voltage was used for studying the size and distribution of the produced nanoparticles.

2.2. Bioluminesence imaging for initial testing of antimicrobial activity

Xenogen IVIS 200 Optical Imaging System (Caliperl Life Sciences, USA) was used for bioluminescence imaging of bacterial growth. The method uses whole bacterial cells as biosensors and is based on the expression of *Photorhabdus luminescens* genes coding for luciferase enzyme in *Escherichia coli*. In the presence of oxygen, luciferase catalyzes light producing reactions that are directly linked to energy balance in cell. The photons are captured in real time by a charge coupled device (CCD) camera in the bioluminescence imaging (BLI) system. This information is converted into figures that express emitted photons or counts on a color scale. Since the amount of emitted light correlates to the well-being of the bacterial strain (Tenhami, Hakkila, & Karp, 2001; Virta et al., 1998), the figures indicate the presence and growth of bacteria.

E. coli K12 carrying a plasmid pCGSL-1 (Frackman, Anhalt, & Nealson, 1990) received from Department of Chemistry and Bioengineering (TUT) was cultured overnight at 30 °C on antibiotic L-agar (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, 15 g/L agar and 200 μg/mL ampicillin). Luminescence was examined with Xenogen and light-emitting colonies were transferred to antibiotic Luria–Bertani medium (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl and 200 μg/mL ampicillin) and incubated overnight at 30 °C

Table 1
Masterbatches and their active ingredient concentrations in coextruded films.

Masterbatch	Active ingredient	Concentration (%) of active ingredient in packaging films
Irgaguard® B5120	Irgaguard B 5000 (silver–zinc zeolite)	2; 3
SG TP8 masterbatch	SG TP8 (silver substituted titanium dioxide)	1