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Starch-halloysite nanocomposites containing nisin: Characterization and inhibition of *Listeria monocytogenes* in soft cheese

Stela Maris Meister Meira [a], Gislene Zehetmeyer [b], Jóice Maria Scheibel [b], Júlia Orlandini Werner [a], Adriano Brandelli [a, *]

[a] Laboratório de Bioquímica e Microbiologia Aplicada, Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, 91501-970, Porto Alegre, RS, Brazil

[b] Instituto de Química, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

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ABSTRACT

Starch/halloysite/nisin nanocomposite films were developed as active antimicrobial packaging. Nanocomposites without nisin and control films (containing starch and glycerol only) were also prepared. Scanning electron microscopy revealed that all samples were homogeneous, halloysite nanotubes (HNT) were dispersed in starch matrix, and film surfaces denoted aggregates as higher amount of nisin was added. X-ray diffraction spectra displayed alterations in typical starch peaks after HNT and nisin incorporation, indicating a decrease in polymer crystallization. Mechanical properties were improved when HNT was incorporated, but inferior values of Young modulus and tensile strength were obtained with nisin addition. Thermal stability decreased for bionanocomposites containing nisin, which showed T_{max} values about 20 °C lower than films without nisin. The antimicrobial activity was tested against *Listeria monocytogenes, Clostridium perfringens* and *Staphylococcus aureus* in skimmed milk agar and all microorganisms were inhibited by nanocomposites containing nisin. These active films were applied on Minas Frescal cheese surface previously inoculated with *L. monocytogenes*. After 4 days, antimicrobial nanocomposite films with 2 g/100 g nisin significantly reduced the initial counts of the bacterium and those with 6 g/100 g nisin completely inhibited *L. monocytogenes*. Results showed that nisin supported in starch/halloysite films can be an active and useful barrier to control food contamination.

1. Introduction

Environmental concerns over non-biodegradable petrochemical-based plastics have raised interest in the use of biopolymers as packaging materials. Among the eco-friendly polymers, starch is one of the most promising candidates since it is easy available, relatively low cost, and renewable natural polysaccharide obtained from a great variety of crops (Gao, Dong, Hou, & Zhang, 2012).

In food industry, when recycling is difficult and/or not economical, especially in short lifetime application, starch films are a possible alternative. Unfortunately, starch presents some drawbacks, such as the strong hydrophilic behavior and poorer mechanical properties than the conventional non-biodegradable plastic films used in food packaging industries (Avella et al., 2005). To circumvent this fact, improvements in the functional properties of these films have been made with nano-sized fillers, such as nanoclays (mainly montmorillonite), resulting in composite materials, namely nanocomposites. Recently, halloysite nanotubes (HNT) become the subject of research attention as a new type of nanofiller for enhancing the mechanical, thermal and the degree of crystallinity of thermoplastic polymers (Schmitt, Prashantha, Soulestin, Lacrampe, & Krawczak, 2012). Moreover, the functionality of packaging can be enhanced by incorporating active substances.

Active packaging mainly comprises the development of films capable of exercising an extra function, in addition to that of providing a protective barrier against external influence. Active packaging is intended to influence the packed food. The active substance that performs a technological function on the food should be authorized as a food additive in the European Union and the use should comply with the applicable restrictions and conditions (EU, 2009). Unlike Europe, no specific legal provisions or

* Corresponding author. ICTA-UFRGS, Av. Bento Gonçalves 9500, 91501-970, Porto Alegre, Brazil.
E-mail address: abrand@ufrgs.br (A. Brandelli).

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regulations deal with active and intelligent packaging materials in the USA. However, several sections of the Food, Drug & Cosmetics (FD&C) Act direct apply to them. The primary applicable regulations on food additives are key legal provisions upon which the Food and Drug Administration relies in determining the safety of active and intelligent packaging (Baughan, 2015). In Brazil, the Resolution RDC 51/2010 sets out general criteria for the determination of total and specific migration of materials, packaging and plastics intended to contact with food (Brasil, 2010).

Preservation of the food from microbial spoilage and contamination/proliferation of pathogenic microorganisms can be achieved by a food packaging material during storage (Dainelli, Gontard, Spyropoulos, Zondervan-van den Beuken, & Tobback, 2008; Meira et al., 2014). *Listeria monocytogenes* is one of the most important food-borne pathogens and many studies have been directed to the use of antimicrobial substances in order to inhibit its growth in food products. Bacteriocins produced by lactic acid bacteria have been often used to inhibit the development of *L. monocytogenes* (Loessner, Guenther, Steffan, & Scherer, 2003; Sobrino-López & Martín-Belloso, 2008). Nisin exhibits antimicrobial activity against Gram-positive vegetative cells like *Listeria* and spores of *Bacilli* and *Clostridia* (Arauz, Jozala, Mazzola, & Vessoni-Penna, 2009), and is recognized as safe by the World Health Organization and approved for use in processed cheese by the Food & Drug Administration. This bacteriocin is used as natural preservative against clostridial spoilage in processed hard and semi-hard cheese, cheese spreads and dairy desserts (Chollet, Sebti, Martial-Gros, & Degraeve, 2008).

Cheese is one ready-to-eat type of food that has been associated with food-borne listeriosis, especially cheeses with high and medium moisture content. Contamination by *L. monocytogenes* is of special concern because of its psychrotrophic nature and its ability to grow at refrigeration temperatures (Gandhi & Chikindas, 2007). This bacterium was previously isolated from Minas Frescal cheese, a fresh, soft, white cheese, which presents high pH (4.9–6.7), high moisture content (>55 g/100 g), and low amount of salt (1.4–1.6 g/100 g) (Brito et al., 2008). Minas Frescal is a typical Brazilian fresh cheese and one of the most highly consumed lactic products in Brazil, with wide acceptance in the national market (Souza & Saad, 2009).

Direct addition of nisin into cheeses results in an immediate reduction of bacterial populations. Meanwhile, if residues of the antimicrobial are rapidly depleted, the antimicrobial will not prevent the recovery of injured cells or the growth of cells that were not destroyed (Chi-Zhang, Yam, & Chikindas, 2004). In this sense, nisin incorporated in antimicrobial nanocomposite films revealed a great potential to reduce post-process contamination by food pathogens (Meira et al. 2014; Salmieri et al. 2014).

Direct melt-extrusion is a more productive and efficient in industrial plants than traditional solvent casting method used for biopolymers preparation. Therefore, the objective of this study was to prepare and characterize for the first time starch/halloysite/nisin nanocomposites by melt-extrusion and further analyze their effectiveness against *L. monocytogenes* in Minas Frescal cheese.

2. Materials and methods

2.1. Materials

A commercial corn starch, Amisol 3408 (Corn Products Brasil, São Paulo, Brazil), was used. The nanoclay halloysite (HNT) was purchased from Sigma–Aldrich (St. Louis, MO, USA). The non-volatile plasticizer was glycerol (99% purity) (Nuclear, Diadema, Brazil). Commercial nisin (Nisaplin®) was provided by Danisco Brasil Ltda (Cotia, Brazil). According to manufacturer, the formulation contains NaCl, denatured milk solids as fillers and 2.5 g/100 g pure nisin.

2.2. Preparation of bionanocomposites

Starch/halloysite nanocomposites were produced in two steps based on Schmitt et al. (2012). The control samples consisted of 75 g/100 g corn starch and 25 g/100 g glycerol and bionanocomposite materials were compounded with 3 and 6 g/100 g nanotubes (named as H3 and H6). Formulations containing nisin were added with two concentrations of the antimicrobial (2 and 6 g/100 g), resulting in four samples designated as H*x*N*y*, *x* taking the value 3 or 6 accordingly to HNT concentration and *y* corresponding to nisin quantity in bionanocomposites.

Components were dried at 40 °C for 48 h prior to physical blend at room temperature. Then, these mixtures were processed by melt mixing using a twin-screw extruder Haake H-25, model Rheomix PTW 16/25, L/D = 25, matrix with L/D = 3 (Thermo Scientific, Karlsruhe, Germany). The temperature setting from the feed to the die was 115–120 °C. After that, the samples were granulated in a Sagec SG-35 (Sagec Máquinas LTDA, Diadema, Brazil) and another extrusion was performed with a profile temperature of 135–140 °C in a extruder Chill Roll AX 16:26 (AX Plásticos, Diadema, Brazil).

2.3. Scanning electron microscopy (SEM)

The film surfaces were analyzed by using a JEOL microscope, model JSM-6060 (Tokyo, Japan) operated at a voltage of 5 kV. To obtain fracture faces, bionanocomposites and the control were cooled in liquid nitrogen, and then broken. Samples were coated with gold layer prior to analysis in order to increase their electrical conductivity.

2.4. X-ray diffraction (XRD)

XRD measurements were performed using a Siemens D-500 diffractometer (Siemens, Karlsruhe, Germany). HNT powder and films were scanned in the reflection mode using an incident Cu K_α radiation ($\lambda = 1.54$ Å), at a step width of 0.05°min^{-1} from $2\theta = 5°$ to 40 °C.

2.5. Fourier transform infrared (FTIR)

FTIR spectra were measured using a Varian 640 IR (Thermo Scientific) spectrometer in attenuated total reflectance (ATR) mode with a diamond crystal. The scans were collected between 600 and 4000 cm^{-1} at a 4 cm^{-1} resolution.

2.6. Mechanical properties

Tensile tests were carried out using films with 20 mm × 70 mm of size using a TA.XT *Plus* Texture Analyzer (Texture Technologies Corp and Stable Micro Systems, Hamilton, MA, USA) according to standard ASTM D-638. The samples were acclimatized for 24 h at 23 °C ± 2 with humidity 50% ± 5 before analysis.

2.7. Thermogravimetric analysis (TGA)

A thermogravimetric analyzer model QA 50 (TA Instruments, New Castle, DE, USA) was used for the thermal stability evaluation. The samples were heated from 25 to 800 C at the rate 10 °C/min under nitrogen atmosphere (50 ml/min).