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Study of nisin adsorption on plasma-treated polymer surfaces for setting up materials with antibacterial properties

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ABSTRACT

Setting up antibacterial materials by nisin adsorption on surfaces depends mainly on the surface properties and the surface treatments allowing the modification of such properties. In order to investigate the factors affecting such adsorption, the native low density polyethylene (LDPE) was modified using Argon/Oxygen (Ar/O_2) plasma, nitrogen (N_2) plasma and plasma-induced grafting of acrylic acid (AA). The films were studied by various characterization techniques. The chemical surface modification was confirmed by X-ray photoelectron spectroscopy (XPS), the wettability of the surfaces was evaluated by contact angle measurements, the surface charge was determined by the zeta potential measurements, and the changes in surface topography and roughness were revealed by atomic force microscopy (AFM). Nisin was adsorbed on the native and the modified surfaces. The antibacterial activity, the nisin adsorbed amount, and the peptide distribution were compared for the four nisin-functionalized films. The highest antibacterial activity was recorded on the Ar/O_2 followed by AA then by N_2 treated films and the lowest activity was on the native film. The observed antibacterial activity was correlated to the type of the surface, hydrophobic and hydrophilic interactions, surface charge, surface topography, nisin adsorbed amount, and nisin distribution on the surfaces.

1. Introduction

The constant lifestyle changes, internet purchasing, globalization of food trade, product shelf-life extension, and demand for natural, minimally processed, and ready-to-eat "fresh" food products present new major challenges for food safety and quality. Antimicrobial packaging materials can provide innovative and promising solutions to such challenges. They can effectively kill or inhibit the growth of micro-organisms that may be present in the packed food or packaging material itself [1]. Such bioactive functional materials can also benefit the biomedical sector for developing antimicrobial implantable devices.

Adsorption of peptides on surfaces can offer a possible way for setting up antibacterial systems. Nisin is a peptide produced by *Lactococcus lactis* subsp. *lactis*. It exerts rapid bactericidal effects against a broad spectrum of Gram-positive bacteria and food pathogens, including *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium botulinum* [2,3]. The bacteriocin has been widely used in the food industry as a safe and natural preservative but has found applications in the biomedical field too [4]. Moreover, it has shown stable activity in the adsorbed state [5]. However, limited information is available on the interactions between the bacteriocin and polymeric materials and peptide adsorption behavior on surfaces has not yet been sufficiently clarified. This behavior is largely controlled by the surface properties (type, composition, charge, topography, roughness, hydrophobic/hydrophilic character...). Therefore, studying those factors is fundamental to understand, control, and improve the adsorption behavior and the antibacterial effectiveness of activated surfaces. Plasma treatments have seen rapid growth in the past decade and can be utilized in many ways for modulating and modifying surface properties of materials [6]. They offer a valuable tool for introducing selectively different functionalities onto polymers [7]; which is required for adsorption and interactions studies. In addition, they are environmentally friendly and can improve the functional properties of inert polymer materials without changing their desirable bulk properties [8].

The objectives of this study were then to evaluate the use of plasma surface modification to study nisin adsorption and antibac-

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terial activity on the functionalized surfaces. Low density polyethylene (LDPE), a well-known polymer in the food and biomedical sectors, was treated with different types of plasma to generate different types of surfaces. Nitrogen (N_2) plasma, Argon/Oxygen (Ar/O_2) plasma, and plasma-induced grafting were used to introduce N-functionalities, O-functionalities, and acrylic acid (AA) monomers to the polymer surface. Nisin was then adsorbed on the native and the three modified materials. The surfaces were characterized by different methods before and after nisin adsorption.

2. Materials and methods

2.1. Materials

Low density polyethylene (LDPE) was obtained from Polimeri Europa (France SAS). Pure water (HPLC grade) and acrylic acid (AA) monomers (99.5%) were supplied from Acros Organics (Belgium). A pure grade of nisin A was donated by Danisco, Beaminster Dorset (United Kingdom). *Listeria innocua* LMG 11387 was provided by BCCM (Belgium) and QBCA reagent by Sigma (France). Brain Heart broth, Mueller Hinton agar medium, and Luria Bertani agar were all purchased from Biokar Diagnostics (France).

2.2. Film preparation

LDPE films (70 μm thicknesses) were cut into 2 × 2 cm^2 and washed with ethanol in an ultrasonic bath to remove possible dusts, oily compounds or any chemicals and wetting agents absorbed on the film surface. They were then dried in an oven at 55 °C for 3 h. Those films were either used directly or treated for nisin adsorption.

2.3. Surface treatments

The native LDPE films were modified using three types of surface treatments: Nitrogen (N_2) plasma, Argon/Oxygen (Ar/O_2) plasma, and plasma-induced grafting of acrylic acid (AA).

Plasma treatments were performed in a radio-frequency cold plasma reactor of 350 l capacity (Europlasma CD1200, Belgium) at an excitation frequency of 13.56 MHz. The preselected vacuum working pressure was 30 mTorr. Experimental designs were set to optimize, for each type of gas, the following plasma process parameters: gas flow rate, generator power, and exposure time. Contact angles values have been taken into account for the optimization using Modde 7.0 software developed by Umetrics (Sweden). The operating conditions used for N_2 plasma were the following: gas flow rate of 500 sccm (standard cubic centimeter per minute), generator power of 300 W, and an exposure time of 300 s. The conditions retained for Ar/O_2 (95/5%) were: gas flow rate of 1000 sccm (standard cubic centimeter per minute), generator power of 420 W, and an exposure time of 245 s. The plasma-induced grafting of acrylic acid (AA) monomers was subsequent to the Ar/O_2 plasma treatment described above, as detailed in our previous work [9]. The amount of grafting on the AA treated film was determined using Toluidine Blue O dye test method [10].

2.4. Surface characterization

2.4.1. X-ray photoelectron spectroscopy (XPS)

XPS experiments were carried out using a Kratos Analytical AXIS Ultra^{DLD} spectrometer (United Kingdom). A monochromatized aluminum source (Al Kα = 1486.6 eV) was used for excitation. The analyzer was operated in constant pass energy of 40 eV using an analysis area of approximately 700 μm × 300 μm. Charge compensation was applied to compensate for the charging effect occurring during the analysis. The C 1s hydrocarbon (285.0 eV) binding energy (BE) was used as internal reference. The spectrometer BE scale was initially calibrated against the Ag $3d_{5/2}$ (368.2 eV) level. Pressure was in the 10^{-10} Torr range during the experiments. Quantification and simulation of the experimental photopeaks were carried out using CasaXPS software. Quantification took into account a non-linear Shirley background subtraction [11].

2.4.2. Contact angle measurements

Static contact angle measurements of the native and treated samples were carried out at room temperature on a Digidrop goniometer (GBX, France) using pure water. A 5 μL drop of water was applied onto the sample surface and the contact angle formed with the surface was instantaneously measured. Triplicate tests were performed for the films and at least six different measurements were performed on each sample surface. The average values for contact angles and the standard deviation were then calculated.

2.4.3. Zeta potential

The zeta potential measurements were performed using a SurPASS Electrokinetic Analyzer (Anton Paar, France) equipped with Ag/AgCl electrodes. The samples were studied inside an adjustable gap cell in contact with the electrolyte (10^{-3} M KCl solution) at the constant value of pH 2, at which nisin adsorption occurred. The measuring cell accommodates two small planar samples with a rectangular size of 20 mm × 10 mm. The sample holders are separated by a 100 μm gap height and form a micro-channel. A pressure ramp from 0 to 300 mbar was employed to force the electrolyte solution through the channel. Before each experiment, an intensive rinsing with the electrolyte solution was done. The zeta potential was calculated from the measured streaming potential using the Helmholtz–Smoluchowski equation and the Fairbrother–Mastin approach [12,13]. Preliminary experiments were carried out for the four types of films to measure the zeta potential versus time. The samples showed generally stable values and the measurement error during each run did not exceed 10%. An average of at least three individual measurements for each sample was reported.

2.4.4. Atomic force microscopy (AFM)

AFM experiments were carried out using a Bruker Dimension 3100 microscope (USA). Topographical images of the films were realized by intermittent contact mode AFM, in air conditions, and at room temperature. In this mode, during scanning over the surface, the cantilever/tip assembly is sinusoidally vibrated by a piezo mounted above it, and the oscillating tip slightly taps the surface. We have used silicon probes with a rectangular cantilever and a tetrahedral tip. The cantilever used is a NCHV-A provided by Bruker, the lever is typically 125 μm long and the apex curvature radius is in the order of 10 nm. The spring constant of the cantilevers and the resonance frequency are respectively 42 N/m and 320 kHz. All images were collected with a resolution of 512 × 512 pixels and a scan rate of 1 Hz on two different regions of the films. Roughness measurements were performed with the Nanotec WSXM software (Spain). The root-mean-squared roughness (RMS) was measured from the analysis of the images at 1 μm × 1 μm scan size. RMS roughness calculation was based on the standard deviation of the Z values, representing the height value in nm between the lowest and the highest point within the given area.

2.5. Nisin preparation

Pure nisin activity was indicated as 5.2 × 10^7 IU/g. Nisin solutions were prepared by dissolving 1.0 mg/ml of nisin in HCl