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Food contact surfaces coated with nitrogen-doped titanium dioxide: effect on *Listeria monocytogenes* survival under different light sources

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ABSTRACT

Improvement of food safety is a very important issue, and is on the basis of production and application of new/modified food contact surfaces. Titanium dioxide (TiO_2) and, more recently, nitrogen-doped titanium dioxide (N-TiO_2) coatings are among the possible forms to enhance food contact surfaces performance in terms of higher hygiene and easier sanitation. In this context, the present work aimed at evaluating the bactericidal activity of an N-TiO_2 coating on glass and stainless steel under two different sources of visible light – fluorescent and incandescent – and ultraviolet (UV) irradiation. *Listeria monocytogenes* was chosen as representative of major foodborne pathogens and its survival was tested on N-TiO_2 coated coupons. In terms of survival percentage, good results were obtained after exposure of coated surfaces to all light types since, apart from the value obtained after exposing glass to fluorescent light (56.3%), survival rates were always below 50%. However, no effective disinfection was obtained, given that for a disinfectant or sanitizing agent to be claimed as effective it needs to be able to promote at least a 3-log reduction of the microbial load, which was not observed for any of the experimental conditions assessed. Even so, UV irradiation was the most successful on eliminating cells on coated surfaces, since the amount of bacteria was reduced to 1.49×10^6 CFU/ml on glass and 2.37×10^7 on stainless steel. In contrast, both visible light sources had only slightly decreased the amount of viable cells, which remained in the range of 8 log CFU/ml. Hence, although some bactericidal effect was accomplished under visible light, UV was the most effective light source on promoting photocatalytic reactions on N-TiO_2 coated coupons and none of the experimental conditions have reached a satisfactory disinfection level. Thus, this surface coating needs further research and improvement in order to become truly effective against foodborne pathogens and, ultimately, become a useful tool towards food safety in general.

1. Introduction

Due to their extremely strong oxidation capability, photocatalytic titanium dioxide (TiO_2) substrates exhibit a self-cleaning function by being able to decompose various types of organic matter [1–3] and also act as disinfectants by injuring both the cell envelope and intracellular components of the microorganisms in contact with those substances. In fact, a cell wall damage followed by cytoplasmic membrane injure leading to a direct intracellular attack has been proposed as the sequence of events when microorganisms undergo TiO_2 photocatalytic challenge [4,5]. This is mostly achieved through the displacement of Ca^{2+}, Na^+ and K^+ ions, which are vital for the bacteria metabolism. Since the microbicidal effect of TiO_2 photocatalytic reactions was reported for the first time in 1985 [6], several works have been done regarding TiO_2 photocatalytic elimination of a wide spectrum of organisms, including bacteria – *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* spp., etc. –, fungi – *Candida albicans*, *Aspergillus niger*, etc. –, algae and cancer cells [4,5,7,8]. Moreover, there are also reports attesting the efficacy of self-cleaning and anti-bacterial TiO_2 coated surfaces, including different material such as glass, sanitary ware, plastic surfaces and food packaging films [9–12], which corroborates the great application potential of this photocatalyst.

Given that TiO_2 photocatalyst is only efficient upon irradiation by ultraviolet (UV) light at levels that would provoke severe injure to human cells, the emergence of nitrogen-doped TiO_2 (N-TiO_2) brought a significant improvement in photocatalytic activity under visible-light [13,14], with an active wavelength range (below 520 nm) covering a wider irradiation energy range for white fluorescent and incandescent light than that of TiO_2 [15]. Inactivation of microorganisms using modified TiO_2 and exposure to visible light has been studied by some researchers, in whose works a significant antibacterial effect was observed inclusively against some pathogens [16–20]. This innovation has risen the potential

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to develop TiO_2-coated surfaces for use in our living environments, which are of particular interest in places where disinfection plays a crucial role in the prevention of infectious diseases, such as hospitals, microbiological laboratories, pharmaceutical industry and food-processing environments. Although fluorescent and incandescent lights are the most commonly used for indoor lighting, and several researchers have used them to study photocatalytic reactions [15,21,22], to the authors' knowledge there is no report concerning the application and performance comparison of both these visible light sources under the same experimental conditions. In this context, the present work aimed at comparing the bactericidal effect of N-TiO_2 coated materials under these two visible light sources and to evaluate the application of this surface treatment on food-contact materials as a way of improving foodborne pathogens control. *Listeria monocytogenes* was the bacterium chosen to represent such microorganisms, as it is responsible for severe food contamination worldwide leading to serious and potentially fatal diseases both in humans and animals. Due to its high efficiency on promoting TiO_2 photocatalysis, and to have comparison between different kinds of light, assays with UV-light irradiation were also performed. Moreover, given that some TiO_2 coatings are known to become super-hydrophilic under UV light irradiation [23–26], surfaces' hydrophobicity was determined through contact angle measurement after exposure to UV-light to verify if this phenomenon occurred on the tested surfaces and, consequently, may have affected surfaces disinfection.

2. Materials and methods

2.1. Coupons with photocatalyst

Stainless steel and glass coupons used in these experiments were coated with N-TiO_2 by pulsed direct current reactive magnetron sputtering, from a high purity Ti target in an Ar/N_2:O_2 atmosphere and subsequently subjected to a post heat treatment at 500 °C in a vacuum furnace. The level of nitrogen doping in the TiO_2 lattice was adjusted by controlling the amount of nitrogen gas in the reactive flow upon sputtering; details of these experiments can be obtained elsewhere [27]. Square glass slides of 2.0 cm × 2.0 cm and stainless steel discs with a 2 cm diameter were used after being cleaned by immersion in a 0.2% solution of a commercial detergent (Sonazol Pril, Alverca, Portugal) followed by immersion in ethanol. Each coupon was then rinsed with ultrapure water and dried at 60 °C. Control coupons had exactly the same characteristics except the coating with N-TiO_2.

2.2. Bacterial culture

For each assay, *L. monocytogenes* clinical isolate 1562 was subcultured on trypticase soy agar (TSA; Merck, Germany) for 24–48 h at 37 °C and then grown in 30 ml of tryptic soy broth (TSB, Merck, Germany) for 18 ± 2 h at room temperature with agitation at 120 rpm. Cells were harvested by centrifugation (5 min, 9000 rpm, 22 °C), washed twice with 0.9% saline and cell suspensions were standardized to an optical density (OD_{640nm}) ≈0.3 corresponding to a concentration of approximately 1×10^9 CFU ml^{-1}.

2.3. Photocatalytic reactions and enumeration of viable bacteria

For each photocatalytic reaction, 50 μl of bacterial suspension were placed on coupon's surface and then covered with a coverslip to improve contact between bacteria and the surface and to prevent the suspension from drying [10]. After optimization of experimental conditions taking into consideration irradiation time and bacterial suspension drying, a 30 min exposure period was selected to perform the assays, which were all done at room temperature (20 ± 2 °C). Three different lights were used - two fluorescent lamps of 4 W each (irradiance of 0.13 mW/cm^2), one incandescent lamp of 60 W (irradiance of 8.93 mW/cm^2) and two UV lamps (irradiance of 0.83 mW/cm^2); the irradiances were measured with a portable photo radiometer (Photo/Radiometer HD 2102.1, Delta Ohm, Italy). The same procedure was conducted for both control and coated coupons. These assays also included coated and non-coated coupons kept in the dark, to be compared with those submitted to irradiation.

After the photocatalytic reactions, surviving bacteria were recovered from each coupon by washing with 1 ml of 0.9% saline. The resultant suspension was serially diluted and the bacterial concentration determined by the standard plating method on TSA plates. Colony forming units (CFUs) were counted after 24 h incubation at 37 °C. At least three independent assays were performed for each material with three coupons per assay.

2.4. Hydrophobicity

The hydrophobicity was determined through contact angle measurement (OCA 20, Dataphysics, Germany) with Millipore water, using the advanced type techique on air. According to this method, a surface is considered hydrophobic if the water contact angle exceeds 65° and hydrophilic if it does not [28]. Measurements were done on glass and stainless steel coupons (coated and non-coated) after 30, 60, 120 and 300 min of UV light exposure, as well as on coupons kept in the dark (controls).

2.5. Statistical analysis

Data analysis was performed using the statistical program SPSS (Statistical Package for the Social Sciences). Contact angle results were compared through one-way ANOVA, whereas bacterial survival was compared using the non-parametric Mann–Whitney *U*-test. All tests were performed with a confidence level of 95%.

3. Results and discussion

In the pursuit of modified surfaces that may enhance sanitation of food processing environments and, ultimately, food safety in general, the photocatalytic capacity of glass and stainless steel (two materials commonly used in kitchens and food processing environments) coated with N-TiO_2 was evaluated under the two light sources most frequently used indoor – fluorescent and incandescent –, as well as under UV-light irradiation. In order to do so, the living organism chosen for this study was the bacterium *L. monocytogenes*, which is one of the major foodborne pathogens nowadays. In this way, the main results of the present work concern bacterial survival under the different conditions tested. Light spectra of the three distinct light types as well as the diffuse reflectance of N-TiO_2 coated materials were also determined. Moreover, the hydrophobicity of all surfaces tested was assessed before and after exposure to the different lights, in order to evaluate if they had influence on such important surface property.

After 30 min of light exposure, bacterial viability was assessed and the number of viable cells compared between the different experimental conditions. Results concerning survival percentages (considering the initial inoculum) after light exposure are presented in Fig. 1, while the correspondent amounts of live bacteria are presented in Table 1. Taking into account that a significant disinfection implies a 3 log reduction of the bacterial load, the more important finding was that none of the tested surfaces had promoted an effective reduction of *L. monocytogenes* survival after 30 min exposure to each light source. On the other hand, in terms of statistically significant differences between the absolute values of CFUs enumeration, it was observed that the number of