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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

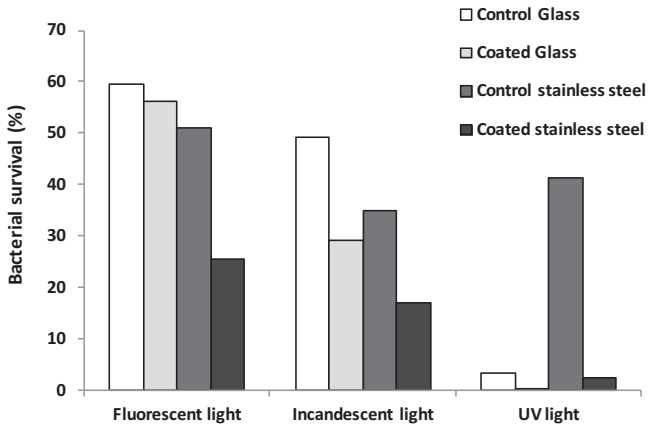

Fig. 1. *L. monocytogenes* percentage survival on control and N-TiO_2 coated glass and stainless steel surfaces after exposure to fluorescent, incandescent and UV light.

viable cells found in the coupons that were kept in dark (data not shown) was not significantly different from the initial inoculum ($\approx 1 \times 10^9$ CFU ml^{-1}), while all other experimental conditions had reduced the bacterial survival on control and coated coupons (Fig. 1). The fact that *L. monocytogenes* survival was reduced in all coupons tested, controls included, may be at least partially due to surface heating during the assays, because of the heat emitted by lamps, given that excessive heating changes the morphological and physiological state of bacteria and, ultimately, can lead to their death [29]. Nevertheless, the significantly lower ($P < 0.05$) survival on uncoated glass exposed to UV-light, comparing to all other controls (Table 1), must be related not only with heating but with the combination of heat and the antimicrobial capability of UV radiation absorbed by glass. In fact, this material absorbs UV-light with greater efficiency than other materials, since electrons in the glass absorb the energy of photons in UV range, in detriment of the weaker energy of photons in the visible light spectrum.

The analysis of the results regarding photocatalytic reactions under visible light revealed a higher effectiveness of the incandescent light, since it had reduced the number of *L. monocytogenes* viable cells on both coated surfaces, while fluorescent light did not accomplish a significant decrease of cell survival on coated glass (Fig. 1). These performances are in agreement with the respective lamp(s) spectra (Fig. 2), which shows that at 380 nm (wavelength below which the photocatalyst's absorbance rapidly increases) fluorescent light has a marginal relative intensity, whereas incandescent light presents a moderate relative intensity. In the same way, UV light efficiency is also corroborated by a higher relative intensity value at 380 nm. Given that the active radiation spectrum of these N-TiO_2 films shows that its major photocatalytic activity occurs on wavelengths below 450 nm (Fig. 3), the better bactericidal performance of incandescent light must be related with its higher relative intensity values comparing to fluorescent light spectrum (Fig. 2).

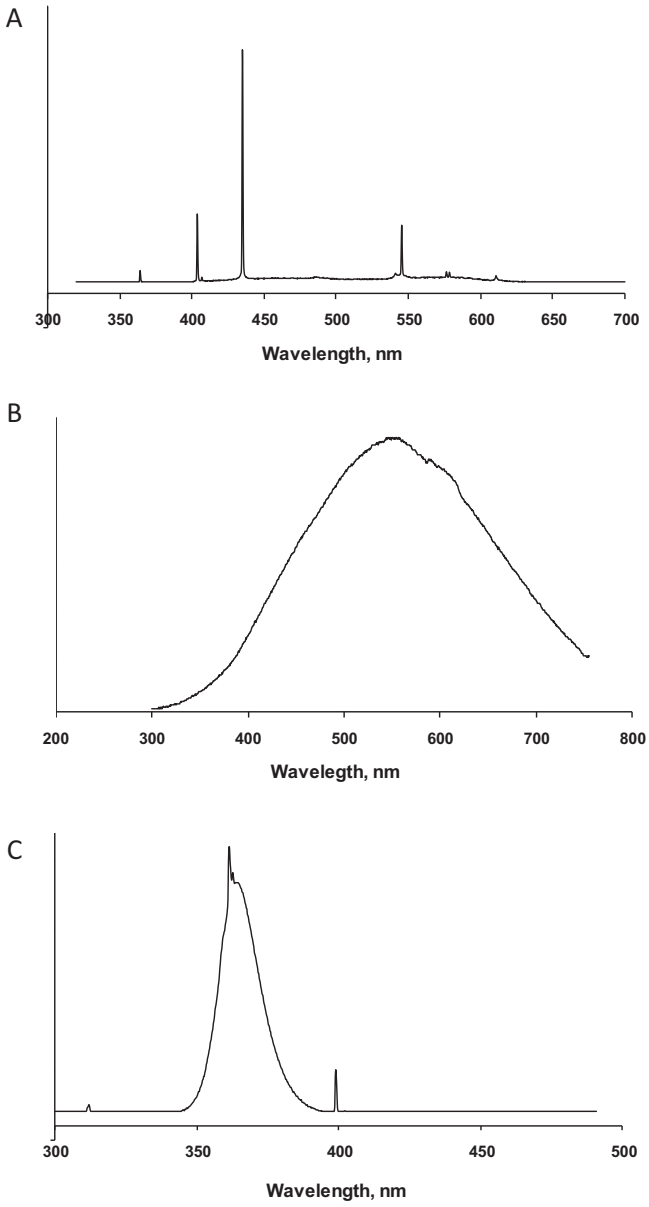

Fig. 2. Light spectra of (A) fluorescent, (B) incandescent and (C) UV lamps.

Considering its features and the results obtained for fluorescent light, it is possible to infer that the statistical disparity on *L. monocytogenes* survival between N-TiO_2 coated glass and stainless steel (Table 1) may be consequence of different interactions between surfaces tested and fluorescent light, where both light and surfaces' characteristics are involved. In fact, analysing the way each material interacts with visible light, diffuse reflectance values showed that

Table 1
L. monocytogenes enumeration after 30 min exposure to different light sources.

Material	Fluorescent light		Incandescent light		UV light	
	CFU/ml	SD	CFU/ml	SD	CFU/ml	SD
Control glass	5.96E+08	1.28E+08	4.92E+08	7.28E+07	3.38E+07†	6.92E+06
Coated glass	5.63E+08	8.42E+07	2.92E+08*,†	1.02E+08	1.49E+06*,†	9.44E+04
Control SS	5.12E+08	1.27E+08	3.50E+08	1.10E+08	4.12E+08	1.00E+08
Coated SS	2.55E+08*	6.59E+07	1.71E+08*	3.77E+07	2.37E+07*,†	7.77E+06

SD – standard deviation.
Symbols indicate statistically different values ($p < 0.05$) between control and coated surfaces of the same material considering the same light irradiation (*) and between the same surface considering different light irradiation (†).

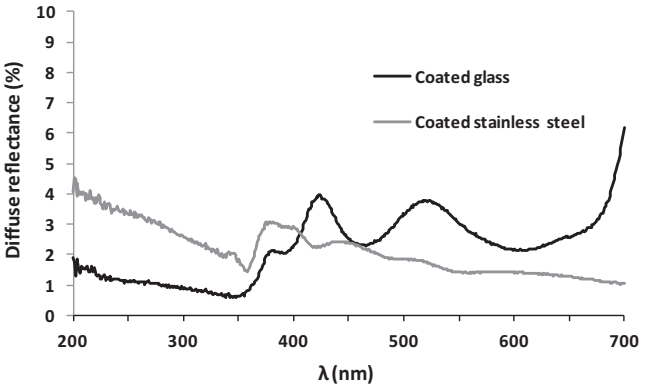

Fig. 3. Diffuse reflectance of N-TiO_2 coated glass and stainless steel.

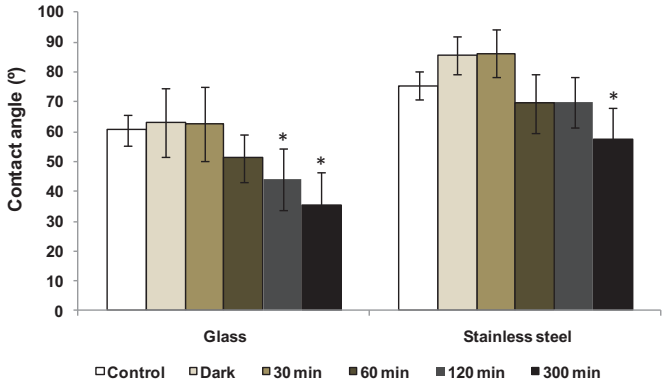

Fig. 4. Water contact angles of uncoated (control) and N-TiO_2 coated glass and stainless steel surfaces at dark and after different exposure times to UV-light. Symbol * indicates statistically different values ($p < 0.05$) between control and coated surfaces of the same material.

the absorption limit of both coated material corresponds approximately to an absorption edge located at ~380 nm (3.26 eV), below which the absorbance rapidly increases. However, for wavelengths above 380 nm, in particular between 400 and 500 nm (visible light range), N-TiO_2 films deposited on stainless steel tend to reflect less diffuse light and absorb more than those deposited on glass (Fig. 3), which explains the better performance of the metal substrate material. It is also worth to notice that, although Morikawa et al. [15] had reported these films to absorb radiation below 520 nm, N-TiO_2 films used in the present work absorb radiation below 450 nm, albeit to a less extent than that is registered below the absorption edge. On the other hand, and still concerning visible light reflectance of both materials, is also important to note that, although both surfaces exhibit a combination of diffuse and specular reflectance, N-TiO_2 films deposited on stainless steel substrates have a higher proportion of specular reflectance and less of diffuse reflectance, in comparison to glass substrates, which inevitably results in a larger dispersion of light on the bacteria and more effective elimination. Specular reflectance implies light rays to be reflected and remain concentrated in a bundle upon leaving the surface, while diffuse reflectance implies the light rays to be reflected and diffused in many different directions. Such different behaviour between the two substrate materials may influence the elimination of bacterial cells in contact with N-TiO_2 films and, thus, contribute to the different results between the two surfaces (Fig. 1). Moreover, fluorescent light was found to emit trace amounts of UV-A, UV-B and UV-C sufficient for bacterial inactivation [30] as well of visible light from the intense discrete peaks at 404 and 435 nm which, all-together, may be the reason why positive bactericidal results on coated stainless steel surfaces were obtained with this light source. Although these surfaces need to be much more developed in order to become truly effective, these results yield hope for the use of such photocatalyst in most indoor environments. It is also important to notice that, taking into account previous studies on the bactericidal efficacy of different kinds of TiO_2 coatings after exposure to UV and/or visible light [21], better results could maybe be achieved with longer periods of light exposure. Nevertheless, it is relevant to remark that most published works on this subject matter tend to present the bactericidal results only as survival percentages [4,10,20], which can easily lead to erroneous conclusions. In fact, as can be confirmed by the results presented in this work, although 1% of bacterial survival may seem a very good result in terms of bactericidal effect, it corresponds to just 2 log reduction of biomass amount, while a 3 log reduction of the bacterial load corresponds to a survival percentage of only 0.1%.

Although results obtained with fluorescent and incandescent light proved that visible light was able to promote some level of *L. monocytogenes* elimination on both materials used, photocatalytic disinfection was significantly higher when UV-light was employed (Table 1). This was already expected due to the disinfection properties of this light, which is patent in the results that showed UV to be the only light that gave significantly different results ($P < 0.05$) between both uncoated materials, with a bacterial survival of only 3.4% on glass and 41.2% on stainless steel (Fig. 1). Results concerning the effect of this light type on coated surfaces are in accordance with Irie et al. [31] that reported N-TiO_2 photocatalytic activity generated by visible light to be inferior to that induced by UV light. Moreover, and in contrast to what happens under visible light, N-TiO_2 and TiO_2 exhibit a similar activity under UV-light [15]. This means that N-TiO_2 photocatalysis under UV-light irradiation has a highly effective bactericidal capability as that reported by many authors concerning TiO_2 photocatalytic reactions [4–8,32–34]. Accordingly, the only efficient photocatalytic reaction found in the present study was accomplished by UV-light irradiation, which achieved the highest levels of disinfection ($P < 0.05$) with *L. monocytogenes* survival as low as 0.1% and 2.4% on N-TiO_2 coated glass and stainless steel, respectively (Fig. 1).

Since there are several reports on TiO_2 coated surfaces becoming super-hydrophilic under UV irradiation [23–26], contact angles were measured in all control and coated surfaces under UV-light irradiation in order to comprehend if that phenomenon was occurring on the materials used in this work and how it could be influencing disinfection. Results are presented in Fig. 4 where, since values of control surfaces were identical for all the conditions tested (at dark and after the different exposure times), only the mean value of those measurements was used and represented in the respective chart. It was observed that both materials coated with N-TiO_2 have a hydrophobic surface and no significant change occurred after UV-light irradiation for 30 min (exposure time used for photocatalytic reactions), which means that UV disinfection performance was not influenced by changes in surfaces' hydrophilicity. Moreover, it took two and five hours exposure, for glass and stainless steel respectively, to find a statistically significant reduction ($P < 0.05$) of hydrophobicity values between controls and coated coupons' (Fig. 4). Moreover, hydrophilicity (contact angle smaller than 65°) was only found in N-TiO_2 coated glass after one, two and five hours UV-light irradiation, while coated stainless steel coupons kept a hydrophobic surface even after those exposure times. The apparent lack of better hydrophilicity of N-TiO_2 coated coupons used in this work is not in agreement with previous reports that found that this coating tends to increase surface hydrophilicity, especially after light exposure [31,35,36]. This disparity might be due to the fact that surface properties are different from those reported in the literature, in particular the surface area of the crystalline grains, which in the present case is very low (<50 m^2/g). It is also important to notice that contact angle measurements on control coupons

and on coated surfaces kept in the dark showed that glass was less hydrophobic than stainless steel (Fig. 4). In this way, and taking into account several studies where less hydrophobicity has been related with less microbial interaction with surfaces [37–40], it is possible to infer that cell–surface interaction was stronger on stainless steel than on glass, which may have enhanced the photocatalytic disinfection performance on the former material.

4. Conclusions

Our results have shown that photocatalytic reactions induced by visible and UV lights on glass and stainless steel surfaces coated with N-TiO_2 were not able to promote an effective disinfection concerning *L. monocytogenes* survival. Nevertheless, some bactericidal effect was achieved by visible light sources, with incandescent light showing a better capacity to promote photocatalytic killing than fluorescent light. In this way, much more work needs to be done in order to improve the bactericidal performance of N-TiO_2 coating surfaces. In fact, if improved and able to promote the desired 3 log reduction of contaminant biomass, this surface coating can became a huge sanitation tool for indoor environments, since it is much safer and cost effective than disinfection using UV and chemical agents.

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