CHITOSAN AS AN ANTIMICROBIAL AGENT FOR FOOTWEAR LEATHER COMPONENTS

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Introduction

Chitosan is a biopolymer obtained by *N*-deacetylation of chitin, which is one of the most abundant natural polysaccharides on earth. Due to several of its intrinsic properties, namely biocompatibility, non-toxicicity, biodegradablility, and also attractive physical and chemical properties, chitosan is being increasingly used in distinct areas such as pharmaceutical, biomedical, cosmetics, food processing and agriculture [1]. Moreover, several biological properties, namely antimicrobial, antitumor, haemostatic, and wound healing properties, have also been reported for chitosan [1,2]. The antimicrobial activity of chitosan against bacteria and fungi has been described by several authors [3] and it is thought to be related to its polycationic structure [1]. Due to this specific characteristic, research concerning the use of chitosan as antimicrobial agent for industrial applications, such as in food, textile and leather industries is presently in focus. In brief, owing to its unique properties, chitosan can pay an important role as an active agent for functional coatings.

In the footwear industry, microorganisms' growth can pose problems of material deterioration with associated unpleasant smell and generate possible infections in susceptible individuals. Generally, footwear presents high relative humidity conditions that enable the growth of bacteria and fungi [4]. Additionally, leather itself, and some tannery agents such as oils and greases, provide a substrate where microorganisms can grow. In the foot, microtraumas caused by ingrown nails, abrasions and lacerations can allow microbial invasion through epidermis, resulting in skin infection [5].

In this work, the applicability of chitosan functional coatings to leather was tested, with the purpose to develop new base materials to produce footwear components. The leather treated with chitosan was then studied for its antibacterial properties against 3 different bacteria.

Material and Methods

Leather treatment with chitosan: Chitosan solution (0.5%, w/v) was prepared by dissolving chitosan (deacetylation degree of 70%) in 2% (v/v) acetic acid and stirring during 1h at 50ºC. The solution was adjusted to pH 5 with NaOH and a leather sample (10 x 10 cm) was dipped in the solution during 30 min at ambient temperature. The sample was washed and dried in a stove at 60ºC.

Antimicrobial activity study: The antimicrobial activity was evaluated using the Agar Diffusion Method based on the AATCC 147 test method [6]. Three different bacteria were assayed including gram positive (*Staphylococcus aureus*) and gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). For each bacteria an inoculum was prepared by transferring four morphological similar colonies from selective media to 10mL of nutritive broth. Each broth was incubated at 35-37ºC (3 to 4 hours, depending on the bacteria) and the obtained suspension was adjusted to 0.5 McFarland standard density. Additionally, the absorbance at 625 nm was recorded for each inoculum. The bacterial solution was then transferred to the surface of a nutrient agar plate by making five consecutive parallel streaks (60mm length, 10mm distance from each other). The leather sample impregnated with chitosan (25 x 50 mm) was then placed transversely across the five streaks and the plate incubated at 37ºC for 24h. Another assay was performed using a sample of leather without chitosan treatment which was used as a control.

Results and Discussion

The used parallel streak method is considered to be useful for estimating antibacterial activity as the growth of the inoculum organism decreases from one streak to the next resulting in increasing degrees of sensitivity [6]. Figure 1 shows the results obtained for the assay using *E. coli*. In both plates, one can observe that the first streak showed smaller inhibition zones than the remaining ones, which can be explained by the higher microorganism concentration in the first streak, and subsequent dilution in the following ones. In the control plate, it could be observed the inexistence of an inhibition zone in the first streak although weak inhibition is seen in the remaining streaks. During leather production different chemical compounds can be used, such as tanning agents and dyes. Possibly, some of those compounds can be retained in minute amounts in the final leather and during the test can spread in the agar, inhibiting the growth of the microorganisms in the less concentrated areas. Compared to control, the leather sample treated with chitosan presented significantly higher inhibition zones which demonstrates the antibacterial effectiveness of the chitosan film tested against *E. coli*. Identical results were obtained in the plates inoculated with *S. aureus* and *P. aeruginosa* showing that the studied chitosan film presented antibacterial activity both against gram-positive and gram-negative bacteria. Nevertheless, *S. aureus* seemed to be the more affected by chitosan film since it showed higher inhibition zones compared to the other two studied bacteria. Possibly, this could be related to the cellular wall differences of gram-positive, such as *S. aureus,* and gram negative bacteria. In fact, other authors have refereed that the antimicrobial activity can be affected by the test organism, with gram-positive being more susceptible to chitosan [1,3].

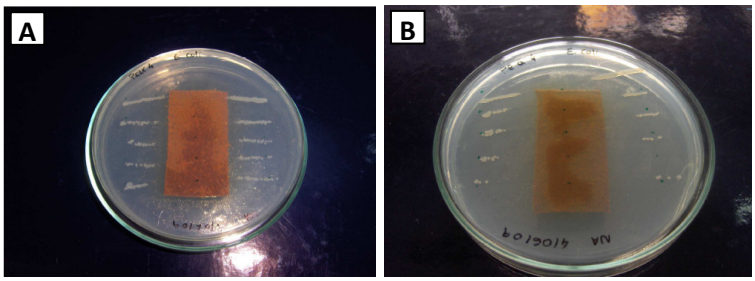

Figure 1 – Paralell streak method using an *E. coli* inoculum; A: control; B: leather treated with 0.5% (w/v) chitosan solution.

Conclusion

The leather treated with chitosan showed antibacterial activity against the three tested bacteria, *E. coli*, *S. aureus* and *P. aeruginosa* thus presenting a high potential to be used as active coating material in prevention and control of bacterial growth in leather footwear components. Considering that several factors have been described to affect the extent of the antimicrobial activity of chitosan, namely molecular weight, deacetylation degree and pH among others, more assays should be performed using chitosan with different characteristics in order to investigate possible increases in antibacterial activity.

Acknowledges

Financial support from COMPETE, QREN and EU (project QREN-ADI-1585-ADVANCEDSHOE) is acknowledge.

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