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Development of chitosan-based antimicrobial leather coatings

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ABSTRACT

The development of antimicrobial coatings for footwear components is of great interest both from industry and consumer's point of view. In this work, antimicrobial leather materials were developed taking advantage of chitosan intrinsic antimicrobial activity and film forming capacity. Considering the specificities of the leather tanning industry, different coating technologies, namely drum, calender and spray, were tested, being the best results achieved with the drum. This last approach was further investigated to assess the effect of chitosan content, type of solubilizing acid, and impregnation time on the achieved antimicrobial capacity. Considering chitosan price (economic reasons) and the obtained results (antimicrobial activity and coating effectiveness, as inspected by SEM), the impregnation in the drum using a chitosan content of 1% (w/v) in a formic acid solution during 2 h, is proposed as the best option for obtaining leather with antimicrobial capacity.

1. Introduction

Nowadays, consumers' concern towards hygiene and active lifestyle is creating new challenges for footwear industry. The intimate contact of the shoe with the foot affords an excellent environment for bacteria and fungi growth owing to the presence of high moisture and warmth conditions, nutrients from feet sweat, and oils/greases from insoles (Orlita, 2004). In footwear, surface colonization and microorganisms' growth can cause material deterioration, generate unpleasant smells (Ara et al., 2006; Sánchez-Navarro, Cuesta-Garrote, Arán-Aís, & Orgilés-Barceló, 2011) and be the focus of infections (Jennings, Alfieri, Kosinski, & Weinberg, 1999). This situation has a major impact on susceptible individuals, such as diabetic patients, which can result in foot ulceration and amputation (Johannesson et al., 2009). Avoiding microorganisms' growth in the feet and footwear is therefore an issue of major interest, both at industrial level and from the consumer's point of view.

To control this problem, antiperspirants and spray formulations containing antibacterial or antifungal substances to be applied directly in the foot or inside the shoe, as well as the use of hygroscopic insoles (Aksoy & Kaplan, 2013) to minimize the presence of water, are the most common commercially available solutions. In what concerns the leather industry, chemical biocides are often used, but mainly to preserve hides during processing rather than to confer antimicrobial properties to the final products (Orlita, 2004; Stockam, Didato, & Hurlow, 2007; TFL, 2013). Moreover, some of these biocides have been restricted or banned due to human health and environmental issues (Sirvaityté, Siugzdaite, & Valeika, 2011; TFL, 2013), and for eco-labelled leathers, limits for certain allowed fungicides are recommended (TFL, 2013).

So far, there are few studies in the literature dealing with the development of antimicrobial leather, and only one addresses footwear applications (Sánchez-Navarro et al., 2011). Those include the use of zinc oxide nanoparticles (Nawaz, Solangi, Zehra, & Nadeem, 2011), surface deposition of silver clusters (Pollini et al., 2013), incorporation of melamine-formaldehyde microcapsules containing tea tree oil (Sánchez-Navarro et al., 2011), treatment with plant essential oils such as eucalyptus and lavender (Sirvaityté et al., 2011) or thyme (Sirvaityté, Siugzdaité, Valeika, & Dambrauskiene, 2012), and coating with polyurethane dispersions added with photoactive antimicrobial agents (Hong & Sun, 2010).

Chitosan, the linear and partly acetylated (1-4)-2-amino-2-deoxy-β-D-glucan isolated from marine chitin (Muzzarelli et al.,

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2012; Muzzarelli, 1977), is being currently proposed for a wide range of applications due to its safety, non-toxicity, biocompability, biodegradability and other unique properties such as film forming capacity and antimicrobial activity (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Muzzarelli et al., 1990; Rinaudo, 2006). Owed to its unique properties, chitosan is considered a viable material for functional coatings development. Its use in the textile industry to develop fabrics with antibacterial activity has already been described with promising results (Dev, Venugopal, Sudha, Deepika, & Ramakrishna, 2009; El-tahlawy, El-bendary, Elhendawy, & Hudson, 2005; Lim & Hudson, 2003; Tseng, Hsu, Wu, & Hsueh, 2009). In the leather industry, examples calling up the use of chitosan and chitosan derivatives can be found but mostly as auxiliary agents in the chrome-tanned leather dyeing process (Aslan, 2013; Burkinshaw & Jarvis, 1996; Plavan, 2012), being the ability to confer antimicrobial capacity only reported as a result of the use of a methacrylic acid–acrylamide–chitosan copolymer (Lv, Yan, & Gao, 2011).

In this work, a novel and alternative solution based on chitosan is purposed to develop antimicrobial leather insoles. The study was conducted in close collaboration with the tanning industry considering three application processes (spray, calender and drum) and the obtained chitosan-coated leathers evaluated for its antibacterial capacity. To the best of our knowledge, chitosan itself was never applied as an antimicrobial agent in the leather industry. Furthermore, the growing consumers' demand towards the use of natural or natural-derived solutions motivates the footwear sector to seek for this type of alternatives.

2. Materials and methods

2.1. Preparation of chitosan solutions

Chitosan 90/200/A1, corresponding to flakes with size < 200 μm, deacetylation degree of 90.5%, and dynamic viscosity of 170 mPa.s (1% at 20 °C in 1% acetic acid solution) from Biolog Biotechnologie GmbH (Germany) was used. Chitosan solutions were prepared by mixing the desired amount of chitosan in acetic or formic acid aqueous solutions (2%, v/v), with stirring overnight (200 rpm at 50 °C). Acetic acid was chosen since it is the most referred in the literature and formic acid due to its use in the tanning industry (dye fixation stage).

2.2. Leather samples coating processes

Leather samples provided by Curtumes Aveneda (Portugal) consisted of several hides, all arising from the same batch, and collected after the dye fixation stage. Different approaches for coating were tested at a pilot scale using the Portuguese Footwear Technological Centre (CTCP) facilities, namely:

(i) Spray-gun (CEI, Companhia de Equipamentos Industriais, Portugal): chitosan solution (1%, w/v, acetic acid) was applied as a finishing step using the spray-gun placed at a distance of 14 cm from the leather sample.
(ii) Calender (Zipor, Equipamentos e Tecnologia Industrial, Portugal): chitosan solution (1%, w/v, acetic acid) was applied using three consecutive passages with the hard pressure rollers maintained at 50 °C and at a distance of 0.90 mm.
(iii) Drum (Zipor, Equipamentos e Tecnologia Industrial, Portugal): impregnations were carried out at 50 °C using half a hide split in two parts with (i) chitosan contents of 0.5%, 1.0% and 3.0% (chitosan/leather ratio (w/w), acetic acid) to evaluate the influence chitosan content; (ii) 1.0% (chitosan/leather ratio, w/w), prepared in 2% (v/v) acetic and formic acid solutions to evaluate the influence of the used type of acid; and (iii) 1.0% (chitosan/leather ratio (w/w), formic acid) during 1 and 2 h to evaluate the influence of the used impregnation time. After the coating process, all the treated samples were dried at industrial conditions and stored in closed plastic bags before antimicrobial activity evaluation. For control purposes, leather samples without chitosan coating were prepared.

2.3. Antimicrobial activity assays

The assays were performed using *Escherichia coli* ATCC 10536. The selection of the used test microorganism follows a preliminary study where chitosan was assayed against Gram positive (*Staphylococcus aureus*) and Gram negative (*E. coli* and *Pseudomonas aeruginosa*) bacteria (Barros, Fernandes, Pinto, Ferreira, & Amaral, 2011). Results showed that *E. coli* was the most resistant bacteria, thus being more conservative to draw conclusions. Two types of assays were used: (i) the Agar Diffusion Method and (ii) the Standard Test Method under Dynamic Contact Conditions. This last test was further modified in order to evaluate the number of consecutive cycles for which an effective antimicrobial activity is achieved. The first test was carried out as a screening assay, thus being performed for all leather samples coated under different processes (spray, calender and drum). The second test under standard conditions was applied to samples arising from calender and drum coating processes, whereas the test under modified conditions was used only with drum samples.

2.3.1. Agar Diffusion Method

This assay was based on the AATCC 147 test method (American Association of Textile Chemists & Colorists, 1998), with minor modifications. Briefly, the bacteria inoculum was prepared by aseptically transferring 4 isolated colonies to nutrient broth, which was then incubated during 24 h at 37 ± 1 °C. The inoculum was diluted to 0.5 McFarland turbidity standard (corresponding to a concentration of 1.5–3.0 × 10^8 CFU/mL) using sterilized Ringer solution. The concentration of this working bacteria dilution was also controlled by spectrophotometry by measuring the absorbance at 625 nm. With a sterilized inoculating loop, five consecutive streaks of 6 cm length each, separated by 1 cm, were made on the surface of a standard Petri dish containing nutrient agar, without refilling the loop. The leather sample (25 mm × 50 mm) was then placed transversely across the five streaks, ensuring intimate contact with the agar surface. The Petri dishes were incubated during 24 h at 37 ± 1 °C. The evaluation of the antimicrobial activity was made based on the presence of an inhibition growth zone around the edges of the tested leather sample.

2.3.2. Standard Test Method under Dynamic Contact Conditions

This assay was performed according to the general guides of ASTM Standard E 2149-01 (American Society for Testing & Materials, 2001). Briefly, the bacteria inoculum was adjusted to 0.5 McFarland turbidity standard as described in Section 2.3.1 and then appropriately diluted with sterile Ringer solution to obtain a final concentration of 1.5–3.0 × 10^8 CFU/mL. This solution was used to prepare the working bacterial dilution employed in the assays by properly dilute it in sterile buffer (0.3 mM KH_2PO_4, pH = 7.2 ± 0.1) to reach a final concentration of 1.5–3.0 × 10^5 CFU/mL. For antimicrobial activity evaluation purposes, a chitosan coated leather sample (2 cm × 2 cm) was introduced in a sterile 250 mL flask containing 50 mL of the working bacterial dilution. The flask was then placed in a bath at 37 °C with orbital stirring. After 1 min under stirring, 1 mL of the solution was aseptically collected to determine bacterial concentration by standard plate count techniques (using serial dilutions and the agar incorporation method in Petri dishes with nutrient agar). The obtained value was considered as bacteria

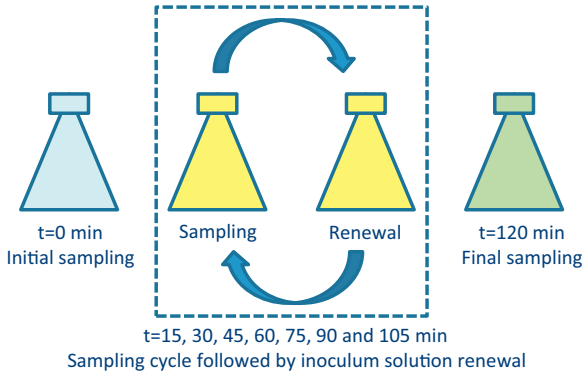

Fig. 1. Schematic representation of the Modified Test Method under Dynamic Contact Conditions.

concentration at the initial contact time (t_0). After sampling, the flask was immediately returned to the bath and stirred for 1 h. After this period, a new sampling of the solution was aseptically collected for bacteria counting. The results of colonies counting were converted to colony forming units per millilitre (CFU/mL) and used to calculate the bacteria reduction in percentage (%). Two other flasks, one containing a leather sample without coating (blank control) and another containing only the working bacterial dilution (without sample addition), were submitted in parallel to the same procedure for colony counting and % of bacteria reduction determination. Considering that after 1 h under stirring complete bacteria elimination (100% reduction) was observed for the chitosan treated leather samples, new assays were performed using narrow sampling periods, namely with solution samples being collected for bacteria counting purposes at 15, 30, 45 and 60 min time periods.

2.3.3. Modified Test Method under Dynamic Contact Conditions

Modifications were introduced in the methodology described in Section 2.3.2, namely a new assay under Dynamic Contact Conditions was performed to evaluate the number of consecutive cycles for which an effective antimicrobial activity is achieved. Briefly, the same working bacterial dilution was used ($1.5–3.0 \times 10^5$ CFU/mL) and bacteria concentration at t_0 determined as described. After 15 min, the leather sample was aseptically removed from the flask and introduced in a new one containing 50 mL of fresh working bacterial dilution, being stirred for more 15 min at 37 °C. The solution from the flask from which the leather sample was removed, was used for colonies counting and calculation of the % of bacteria reduction. This procedure was repeated, with inoculum renovation every 15 min, until a total period of 120 min was reached (Fig. 1). All bacteria counting (CFU/mL) was performed in nutrient agar, after incubation for 24 h at 37 °C.

For a given contact time, the percentage of colony number reduction was determined according to the following equation:

$$\text{Reduction}\,(\%)\,(\text{CFU/mL}) = \left(\frac{B-A}{B}\right) \times 100$$

where A is colony counting for the flask containing the sample and B the colony counting for the flask containing the inoculum solution alone.

2.4. Scanning electron microscopy (SEM)

Morphological modifications of the leather sample surface due to chitosan coating were evaluated through scanning electron microscopy (SEM) with a Phenom G2 Pro microscope (Phenom-World, Eindhoven, The Netherlands) equipped with a backscattered electron detector (BSE) operating at 5 kV. Samples were analyzed under different magnifications using a Charge Reduction Sample Holder without any previous sample preparation.

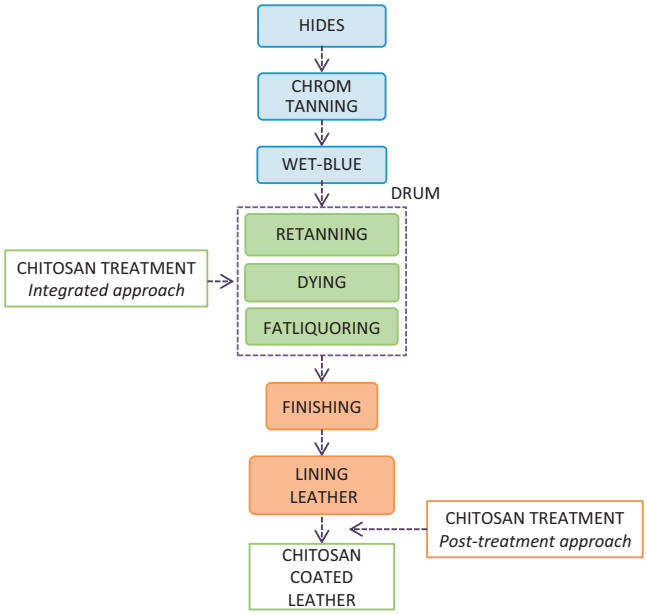

Fig. 2. Schematic representation of the global process main steps used in the leather tanning industry.

2.5. Evaluation of colour fastness to mechanical stress and perspiration

The degree of damage and transfer of leather surface colour during mild dry or wet abrasion was evaluated following the ISO 17700:2004 standard (ISO, 2008). Briefly, leather samples were rubbed with dry or wet wool felt pad during 512 or 256 cycles, respectively. The evaluation was made by using a geometric grey scale.

The colour fastness to perspiration was evaluated following ISO 11641:2012 standard (ISO, 2012). Briefly, a leather sample and a piece of a multifibre adjacent fabric (wool, acrylic, polyester, nylon, cotton and cellulose acetate) were immersed separately in an artificial perspiration solution and set under vacuum. After being removed, the fabric was covered with the leather sample and then placed between two glass plates and pressed (180 min at 37 ± 2 °C). Once dried, the leather colour changes as well as the staining of multifibre adjacent fabric were assessed by using a geometric grey scale.

3. Results and discussion

3.1. Evaluation of drum, calender and spray treatments

Considering the global process used in the leather tanning industry, the development of chitosan-based antimicrobial coatings could be performed based on two different approaches, namely as an integrated treatment (i.e., as additional step to be included in the tanning process) or as a post-treatment of the lining leather (Fig. 2). In the first case, since chitosan is soluble in acidic aqueous solutions due to protonation of its amino groups, and considering that the dye fixation stage is generally carried out in formic acid medium, an interesting solution would be the integration of an additional step for chitosan leather impregnation after the dye fixation stage (before fat liquoring) where the process conditions (including pH) are adequate. Considering that during dye fixation stage the leather inside the drum is already under

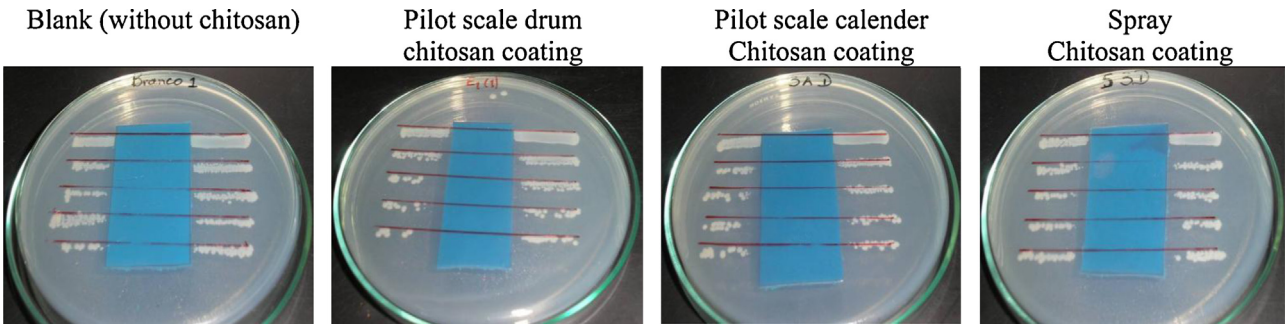

Fig. 3. Screening evaluation of antimicrobial activity using the Agar Diffusion Method.

acidic pH, chitosan coating only requires the addition of chitosan solution, thus being simple to execute and not troublesome for the whole tanning process. This last aspect is considered a very important feature from the tanning industry's point of view. The second possible approach, the post-treatment strategy, is also considered as being simple to execute and thus also feasible to be applied at industrial level. Moreover it could be applied to produce small size and customized series.

In this work, both strategies for chitosan leather coating were evaluated at a pilot scale: the integrated treatment by using a pilot scale drum and the post treatment approach by using a pilot scale calender and an industrial spray-gun. To compare the effectiveness of both approaches, leather samples were coated using a 1.0% chitosan solution prepared with acetic acid, and evaluated for its antimicrobial activity using the Agar Diffusion Method (Fig. 3). As it can be observed, compared to the blank control (leather without chitosan treatment), all the treated samples showed the existence of bacteria growth inhibition. This result indicates that chitosan coating was successfully achieved for all the evaluated approaches, as expected since at acidic pH chitosan amino groups become protonated, thus readily binding to negatively charged groups and behaving like a bioadhesive (Torres-Giner, Ocio, & Lagaron, 2008). However, results showed smaller inhibition zones in the spray treated sample when compared to the calender and drum samples suggesting that this treatment was the less effective. This can possibly be explained by a more superficial application of the chitosan solution resulting in a less efficient leather impregnation. Moreover, spray application gave rise to products showing stains and gloss (see Fig. 3 – spray chitosan coating) which is not acceptable from an industrial point of view, thus limiting its use. By the contrary, leathers coated in the drum did not showed this problem, the same happening with calendering, which consequently presented a higher potential as post-treatment comparatively to spray application. Based on these results, the spray post-treatment approach was discarded.

Since similar antimicrobial activity was observed in the screening test for both leathers impregnated using the calender and the drum, the samples were further tested under Dynamic Contact Conditions. Initially, the evaluation of the antimicrobial activity was performed according to the general guides of ASTM Standard E 2149-0, with solution sampling for colony counting being performed after 1 h of contact of the leather sample with bacteria inoculum. In both cases (calender and drum coated leathers) a considerable antimicrobial activity was observed since there was no microorganism's growth on the Petri dishes reflecting a bactericidal effect corresponding to 100% bacterial reduction. Subsequently, the test was repeated using shorter sampling periods (being performed each 15 min). Results for colony counting and corresponding % of bacteria reduction are shown in Table 1. As it can be observed, the leather sample submitted to chitosan coating in the drum evidenced a strong antimicrobial activity showing no bacteria growth for the sampling performed at 15 min. Regarding the leather submitted to calender treatment, only after 45 min of close contact of the sample with the bacteria inoculum, a total bacteria reduction (100%) was achieved, thus exhibiting a lower antimicrobial activity when compared to the corresponding drum treatment. Decrease of bacteria counting (CFU/mL) was also observed in the blank sample (leather without chitosan treatment) along the test period, although complete reduction (100%) was not achieved for the 60 min sampling. This can be related to the fact that the leather used in this experiment arrived to the Portuguese Footwear Centre directly from the tanning industry, still immersed in the dying solution. The leather sample used as the blank control was simply washed with deionized water before being dried. Therefore, a possible explanation could be the presence of residual substances remaining from the tanning process, such as the dye itself and formic acid, which can present some antimicrobial activity.

Analysing the results regarding antimicrobial activity presented by the different evaluated leather samples, all coated with 1% chitosan in acetic acid solution, the treatment corresponding to chitosan impregnation performed in the drum, which in addition can support the development of an integrated process, was chosen as the most promising approach.

3.2. Chitosan-coating using a pilot scale drum: effect of chitosan content, type of acid and impregnation time on antimicrobial activity

To evaluate the effect of chitosan content (0.5%, 1% and 3%), type of acid (acetic and formic acids), and impregnation time (1 and 2 h) on the antimicrobial capacity of the chitosan-based coatings produced in the pilot scale drum, a set of coating experiments under the mentioned conditions have been performed. As a first approach to evaluate the antimicrobial activity, the Agar Diffusion Method was used as a screening test.

By visually inspecting the inhibition zones displayed around the samples coated using solutions with the same amount of chitosan but prepared with different acids (formic or acetic), no significant differences were noticed (data not showed). Therefore, formic acid is a suitable solution since it is already used in the industrial leather tanning process during the dye fixation stage, thus simplifying the introduction of chitosan in the whole process. Regarding chitosan amount (0.5%, 1% and 3%), results of the screening test showed differences among the samples; with the 0.5% chitosan coating presenting the lowest antimicrobial activity, as expected. Samples coated with 1% and 3% have showed larger inhibition zones; nonetheless they were similar between each other (data not shown).

According to these results, leather samples coated with 1% and 3% chitosan in formic acid were chosen to be further assayed using the Dynamic Contact Conditions. Since coating with 1% chitosan was considered more interesting and industrially feasible, than

Table 1
Results for the Test method under Dynamic Contact Conditions (colony counting expressed as mean ± standard deviation and corresponding % of bacteria reduction). Leather samples were coated using 1% chitosan solution in acetic acid.

Time (min)	Sample								
	Leather without chitosan (blank)			Drum chitosan-coated leather			Calender chitosan-coated leather		
	Inoculum (CFU/mL)	Leather sample (CFU/mL)	Bacteria reduction (%)	Inoculum (CFU/mL)	Leather sample (CFU/mL)	Bacteria reduction (%)	Inoculum (CFU/mL)	Leather sample (CFU/mL)	Bacteria reduction (%)
0	$(2.70 \pm 0.01) \times 10^5$	$(1.96 \pm 0.09) \times 10^5$	27.4	$(3.00 \pm 0.001) \times 10^5$	$(1.90 \pm 0.03) \times 10^5$	36.7	$(2.10 \pm 0.07) \times 10^5$	$(2.06 \pm 0.06) \times 10^5$	1.9
15	$(2.67 \pm 0.01) \times 10^5$	$(1.61 \pm 0.06) \times 10^5$	39.7	$(3.00 \pm 0.01) \times 10^5$	–	100.0	$(2.01 \pm 0.01) \times 10^5$	$(6.50 \pm 0.05) \times 10^4$	67.7
30	$(1.81 \pm 0.003) \times 10^5$	$(9.10 \pm 0.06) \times 10^4$	49.7	$(2.10 \pm 0.01) \times 10^5$	–	100.0	$(1.78 \pm 0.06) \times 10^5$	$(3.40 \pm 0.04) \times 10^3$	98.1
45	$(2.27 \pm 0.01) \times 10^5$	$(2.90 \pm 0.04) \times 10^3$	98.7	$(3.00 \pm 0.001) \times 10^5$	–	100.0	$(1.91 \pm 0.02) \times 10^5$	–	100.0
60	$(1.83 \pm 0.002) \times 10^5$	$(6.00 \pm 0.02) \times 10^2$	99.7	$(2.58 \pm 0.02) \times 10^5$	–	100.0	$(1.73 \pm 0.04) \times 10^5$	–	100.0

Table 2
Results obtained in the Modified Test Method under Dynamic Contact Conditions (with inoculum renewal).

Time (min)	Bacteria reduction (%)[a]		
	1% chitosan 1 h impregnation	1% chitosan 2 h impregnation	3% chitosan 1 h impregnation
15	94.3	60.7	57.7
30	98.3	99.6	99.5
45	96.6	100.0	100.0
60	97.4	100.0	100.0
75	95.1	99.0	98.5
90	91.9	98.8	98.0
105	89.5	81.7	78.7
120	50.4	37.8	27.4
Renewal cycles with bacteriostatic effect[b]	6	5	5
Renewal cycles with bactericidal effect[b]	0	2	2

[a] Using the equation presented in Section 2.3.2.
[b] Bacteriostatic and bactericidal effects were considered when a reduction between 90% and 99.9% and a reduction ≥ 99.9%, respectively, were achieved.

using 3% in view of economic costs, a sample coated with the same 1% chitosan content but for which a 2 h impregnation in the drum was used, was also assayed. Considering that for the initial experiments, the leather samples coated in the drum exhibited a high antimicrobial activity against *E. coli*, showing a 100% bacteria reduction after 15 min of contact, the Modified Test Method as described in Section 2.3.3 was applied. This modified test included a bacteria inoculum renewal, meaning that every 15 min a sampling for colony counting was made and the leather sample withdrawn and put in a new inoculum solution. Table 2 shows a survey of the obtained results for the three evaluated leather samples, evidencing the bacteria reduction (%) after each inoculum renewal cycle, calculated after pour plating and bacteria counting. Table 2 also includes information concerning the total number of renewal cycles where bacteriostatic and bactericidal effects were observed. Bactericidal activity was considered when a reduction ≥ 99.9% (or ≥ $3 \log_{10}$) of the total count of CFU/mL in the original inoculum was achieved, as defined by the former National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 1999). Bacteriostatic activity was considered when a reduction between 90% and 99.9% of the total bacteria count (CFU/mL) in the original inoculum was achieved (Pankey & Sabath, 2004). All the assayed samples showed high antimicrobial activity, as evidenced by bacteria reduction even after 8 renewal cycles, thus pointing to the viability of using chitosan to confer antimicrobial properties to leather. Regarding the used chitosan amount, the 3% coated sample evidenced an antimicrobial activity similar to the ones coated with 1%. In view of chitosan price, that is considered high in the context of the tanning industry, and the obtained results, the use of 1% chitosan content for leather coating is advantageous over the use of 3%, constituting a better compromise for the tanning industry. In what concerns the parameter "impregnation time", the two samples also showed similar results (Table 2). Nevertheless, for samples impregnated in the drum during 2 h a higher number of renewal cycles where bactericidal effect is effective were achieved comparatively with the 1 h impregnation time.

In order to assess leather suitability for end use, an evaluation of colour fastness to rubbing and to perspiration is generally performed. Thus, the leather sample that exhibited the higher antimicrobial activity, namely the one coated during 2 h in the drum using a 1% chitosan solution in formic acid, was evaluated for colour fastness. The obtained results for the chitosan coated leather were very good to excellent (4/5-5 using the geometric grey scale) for all the performed tests and identical to the ones obtained with the

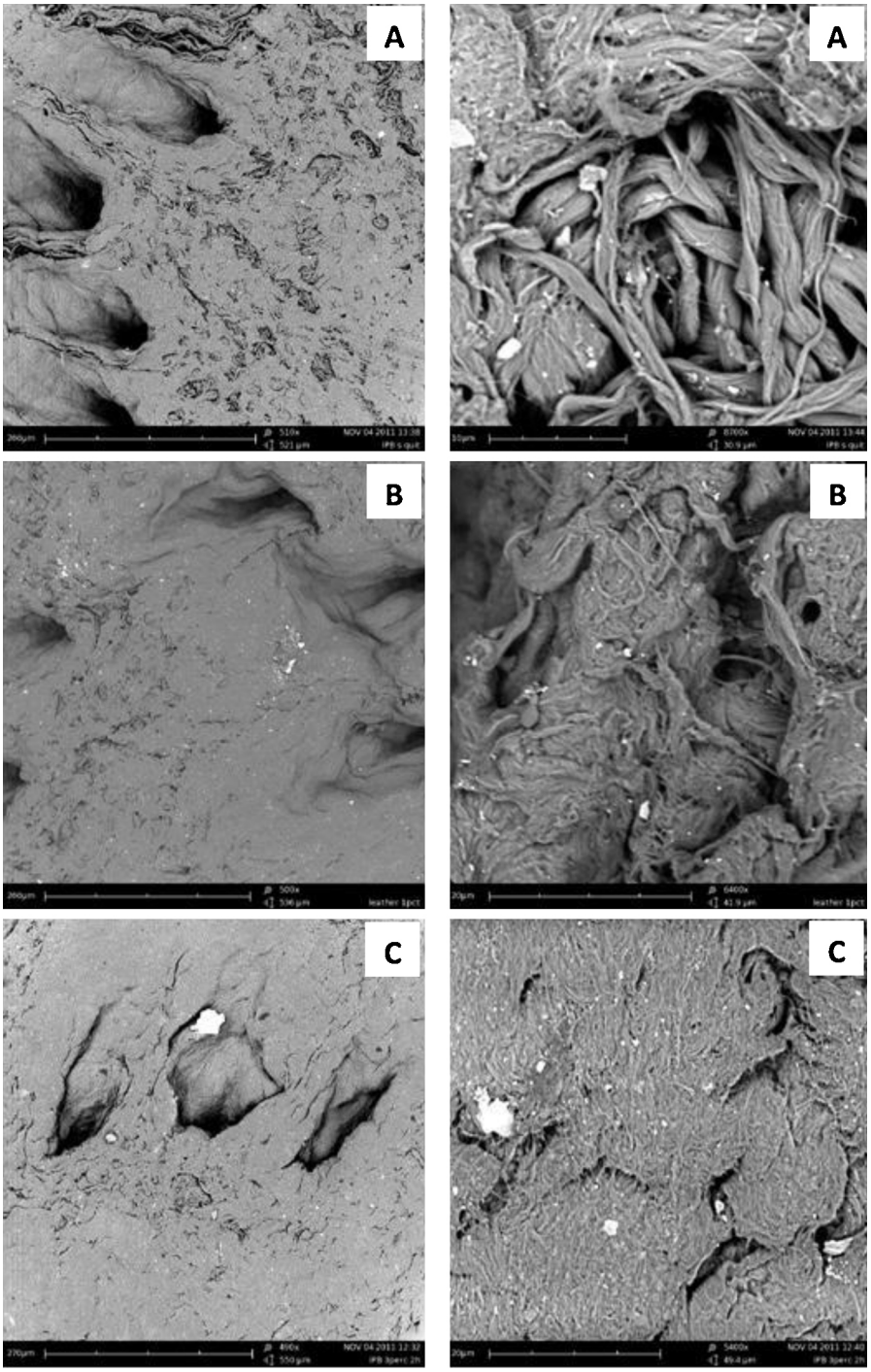

Fig. 4. SEM images of the non-coated leather (A) and after being submitted to chitosan impregnation in the pilot scale drum: 1% chitosan content (formic acid) (B), 3% chitosan content (formic acid) (C).

control sample (same leather without coating), thus in conformity with the ISO standards requirements. Results showed that chitosan coating did not increase the likelihood of colour bleeding from the material being suited to be used in footwear components.

3.3. Scanning electron microscopy (SEM)

Samples impregnated in the pilot scale drum using 1% and 3% chitosan contents and formic acid solutions, as well as a blank (leather sample without chitosan impregnation), were evaluated through scanning electron microscopy to check for morphological surface differences (Fig. 4) and thus evaluate the impregnation process effectiveness.

The images revealed an effective chitosan deposition in the leathers impregnated in the drum (Fig. 4B and C), being the results consistent with the antimicrobial activity evaluation tests. Comparatively to the images in Fig. 4A showing the leather without chitosan coating, both Fig. 4B and C evidence a more homogeneous and smoother surface with less fibbers being visible, i.e., uncoated, which can be attributed to the formation of an active chitosan film coating covering the leather sample. In general, SEM observations are in good agreement with the results presented in Table 2.

Only slight differences are observed between chitosan coating films aspect when using 1% and 3% chitosan contents. This means that 1% chitosan content is enough to obtain an effective coverage of the leather sample.

4. Conclusions

In this study, different approaches for leather chitosan-coating were evaluated aiming to develop leather with antimicrobial properties to be used in footwear components. Two main solutions were assayed, namely an integrated approach corresponding to an impregnation performed as an additional step after the dying stage (tested using a pilot scale drum), and a post-treatment approach where the coating is applied as a finishing step either using a spray-gun or a calender. Considering the obtained results it is clear that chitosan coating conferred antimicrobial properties to the treated leathers, with the higher capacity to eliminate *E. coli* being achieved when the drum technology is used. Other advantage of the integrated treatment approach is that the dye fixation stage already comprises the use of acidic solutions, which simplifies the introduction of this new stage (addition of chitosan solution) in the whole process. Among the evaluated options, coating using 1% chitosan content (formic acid solution) performed in the drum during 2 h, is proposed as the best solution to be used by the tanning industry considering both antimicrobial efficacy and economic benefits.

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