

COMFORT AND ANTIMICROBIAL PROPERTIES OF DEVELOPED BAMBOO, POLYESTER AND COTTON KNITTED SPACER FABRICS

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3D textile structures with different fibre composition were produced and evaluated in terms of breathability, thermal comfort and antimicrobial activity. Bamboo, cotton, polyester and bioactive polyester were used in proportions ranging from 14 to 72% for the fabrics. Out of the developed materials showing good mechanical and comfort properties, the knitted spacer fabric made up of bamboo and polyester exhibited the best global performance. The results of antimicrobial tests revealed the importance of contact area and type of bioactive agent for antimicrobial protection. Knitted spacer fabrics with 72% antimicrobial fibres, such as bamboo, were not active against the microorganisms tested, whereas knitted flattened fabrics made with the same yarn were effective against *E. coli*, *P. aeruginosa* and *T. rubrum*. The incorporation of thyme oil into the textiles imparted activity against *E. coli*, *S. aureus*, *C. albicans* and *T. rubrum*. The developed textiles have the potential to be used as footwear lining to prevent athlete's foot and associated bacterial infections.

Keywords: antibacterial, antifungal, 3D spacer textiles, comfort properties

INTRODUCTION

Dermatophyte infections affect 20 to 25% of the world populations and constitute a serious public health problem. Dermatophyte fungi metabolize the keratin of human epidermis, hair and nails. *Tinea pedis*, also known as athlete's foot, is a form of ringworm, a highly contagious skin infection associated with fungi, including *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Fungal infections of the nails, known as onychomycosis, account for 50% of all nail infections and become more common with advancing age. The problem is even more severe for some risk patient groups, with uncontrolled diabetes, AIDS, renal diseases, psoriasis, and types of immunosuppression, such as transplant recipients and patients on long-term corticosteroid therapy. Other risk factors include nail trauma, frequent immersion in water and occlusive footwear. In 70% of the cases, onychomycosis is also caused by dermatophytes, namely *T. rubrum* and *T. mentagrophytes*, and causes pain,

discomfort, itching and burning sensations on the feet, as well as malodour. Yeasts such as *Candida albicans* and *Candida parapsilosis* are also relevant infectious agents primarily in fingernails. These infections are associated with a subversion of the host's inflammatory/immune responses that facilitate the onset and progression of the infection. Besides inflammation, the damaged tissue becomes more vulnerable to commensal bacteria that become pathogenic. In these infections, bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* are also relevant.¹⁻²

Pharmacological treatment of *Tinea pedis* and onychomycosis usually involves the use of topical and oral antifungal agents, such as terbinafine, clotrimazole, miconazole, fluconazole, or ciclopirox creams. However, research data has shown that such treatments fail to cure about one-third of patients with *Tinea pedis*. Fungi in shoes or exposure to certain environments cause most of

the relapses. Thus, it is necessary to develop products with antifungal/antibacterial properties for shoes, especially for the treatment of *Tinea pedis* and common bacterial species present in co-infections.² Alternatives based on natural compounds, such as phenolics, essential oils and extracts have been proposed.³

The advantages and disadvantages of the use of antimicrobial agents for textile materials have been discussed thoroughly.⁵⁻⁶ They differ in their chemical structure, effectiveness, application process, and human health promoting properties.⁷⁻

⁸ The non-leaching approach is attractive for textile applications, especially if the durability and safety is a mandatory requirement. However, leaching antimicrobials could be interesting for textile applications based on 3D matrix without washing fastness requirements. Recently, in order to produce biocompatible and eco-friendly bioactive textiles that do not promote resistant pathogens, natural derivatives have attracted attention. Thus, plant-based antimicrobial agents have been increasingly used for textile bio-functionalization. These antimicrobials include phenolic and polyphenols terpenoids, alkaloids, lectins, polypeptides and essential oils.⁹⁻¹³

Thyme (*Thymus* spp.) is a widely available Lamiaceae and its essential oils are applied in several traditional uses, especially as antiseptic. The essential oil of *Thymus vulgaris* has been claimed as antimicrobial. *Thymus vulgaris*, *Thymus pulegioides* and *Thymbra capitata* essential oils, with high content of thymol and/or carvacrol, showed antifungal activity primarily against dermatophytes in comparison with *Candida* species. The antidermatophytic effects of carvacrol and thymol were also evaluated and proved to be equivalent to that of the essential oil, thymol being more active than carvacrol.¹⁴⁻¹⁶ However, other authors reported that thymol presented a 30 times higher antimicrobial activity and 4 times lower toxicity than carvacrol.¹⁷⁻¹⁸ Also, De Smet *et al.*⁹ showed that cotton knitted fabric immersed in a thymol and carvacrol solution had a significant activity towards *S. aureus* and *Escherichia coli*.

Research on antimicrobial properties of fabrics or fibres functionalized with different agents has dealt mainly with antibacterial properties, whereas antifungal activity has received much less attention. Also, many of these products may not be suitable for patients with *Tinea pedis*. A variety of chemical finishes have been used to produce textiles with noticeable antimicrobial

properties. The application process depends on the type of antimicrobial agent and textile fibre, the use of the materials, the fabric construction and the required level of fixation. The incorporation of antimicrobial agents into textiles can be made by simple impregnation methods or by more complex grafting and crosslinking processes, but with a common target to retain comfort and mechanical properties.^{9,20-25}

Furthermore, the use of bioactive plant-based products and sustainable biopolymers presents a novel opportunity for large-scale development of bioactive textiles.²¹⁻²³ Some natural fibres, such as flax, bamboo, hemp and kapok, are particularly interesting since they impart antimicrobial activity.¹² Footwear and textile industries prioritize not only product quality and design, but also customer comfort. With regard to foot comfort, the adaptation of the textile lining material to foot movements, breathability, thermal and moisture control and friction are the main parameters that need to be taken into account in the development of textiles for shoes. Thus, the nature and structure of materials are key parameters to design comfortable shoes. Additionally, antifungal/antibacterial properties add hygienic and therapeutic characteristics. This paper aims to discuss the structure of textiles and the antimicrobial functionalization method to achieve sustainable materials for use as footwear lining. Different 3D textile structures were produced with mixtures of bamboo, cotton and polyester fibres. Comfort related properties, such as air permeability, water vapour permeability and thermal properties, as well as the antimicrobial activity of the obtained textiles were analysed.

EXPERIMENTAL

Textile materials

Cotton (19.7 Tex), bamboo (24.6 Tex), polyester (19.7 Tex), and bioactive polyester (49.2 Tex) yarns were used to produce different textile structures. Several knitted warp spacer fabrics were produced on a flat knitting machine (Stoll CMS 320 TC E5, H. Stoll GmbH & Co. KG, Reutlingen, Germany). The overall composition of samples 1 to 8 and their relative composition by face can be seen in Table 1.

Bamboo viscose fibre, bioactive polyester, and cotton yarns were used to produce knitted jersey structures (spacer and flattened fabrics) with different porosities. Throughout this paper, the spacer fabrics developed are referred to by their numbers (1 to 8 as presented in Table 1) and flattened fabrics as samples 9 (bamboo viscose, 100%), 10 (bioactive polyester, 100%) and 11 (cotton, 100%).

Table 1
Composition of knitted spacer fabrics

Sample	Face	Cotton (%)		Polyester (%)		Bioactive polyester (%)		Bamboo (%)	
		By face	Overall	By face	Overall	By face	Overall	By face	Overall
1	A	0		0		100		0	
	B	100	28	0	0	0	72	0	0
	Spacer	0		0		100		0	
2	A	0		0		100		0	
	B	100	61	0	0	0	39	0	0
	Spacer	100		0		0		0	
3	A	50		50		0		0	
	B	0	14	0	14	0	0	100	72
	Spacer	0		0		0		100	
4	A	0		0		0		100	
	B	0	28	100	32	0	0	0	40
	Spacer	100		0		0		0	
5	A	0		0		0		100	
	B	0	0	100	30	0	0	0	70
	Spacer	0		0		0		100	
6	A	0		0		0		100	
	B	0	0	100	61	0	0	0	39
	Spacer	0		100		0		0	
7	A	0		0		100		0	
	B	100	44	0	0	0	37	0	18
	Spacer	44		0		0		56	
8	A	0		0		100		0	
	B	100	28	0	0	0	36	0	36
	Spacer	0		0		0		100	

Thyme essential oil: determination of minimal inhibitory concentration (MIC) of *T. vulgaris* and application on fabrics

Thyme oil, containing as main components, thymol (44.88%), *p*-cymene (20.53%), carvacrol (4.60%), linalool (5.98%) and γ -terpinene (14.27%), was supplied by Soria Natural (Portugal). All other reagents were of analytical grade and supplied by Sigma-Aldrich (Spain).

The minimal inhibitory concentration (MIC) for *T. vulgaris* was evaluated according to CLSI standard tests for bacteria, yeast and fungi, M07-A9, M27-A2 and M38-A2, respectively.

Dry fabrics, without antimicrobial activity were impregnated by immersion in different concentrations of the essential oil, from 0.32 to 5 $\mu\text{L mL}^{-1}$. Reference samples were treated with solvent only. Samples were placed in individual sterile Petri dishes and dried for 24 h at 37 °C.

Samples characterization

The textile materials were characterised in terms of weight (mass per unit area), porosity, thickness, air permeability, water vapour permeability and thermal properties (thermal conductivity, thermal resistance and thermal absorptivity).

All the tests were carried out after conditioning the samples under standard atmospheric conditions (20 \pm 2

°C and 65 \pm 2% relative humidity), in accordance with the conditions defined by standard ISO 139:1973.

Mass per unit area (weight)

The mass per unit area was determined according to the procedure described in standard NP EN 12127:1999.

Thickness

The thickness of the samples was determined according to the procedure described in standard EN ISO 5084:1999.

Apparent density

Apparent density was calculated using the following equation, described by Blaga *et al.*,³⁰ where *M* is mass per unity area and *T* is thickness:

$$\text{Density (gm}^{-3}\text{)} = \frac{M \text{ (gm}^{-2}\text{)}}{T \text{ (m)}} \quad (1)$$

Porosity

Porosity (%) was calculated using the following equation, as described by Rajan *et al.*¹³ and Ghorbani *et al.*,¹⁴ where ρ_{fabric} is fabric density and ρ_{fibre} represents fibre density:

$$\text{Porosity (\%)} = 1 - \frac{\rho_{\text{fabric}} \text{ (gcm}^{-3}\text{)}}{\rho_{\text{fibre}} \text{ (gcm}^{-3}\text{)}} \quad (2)$$

Air permeability

Air permeability was determined according to the procedures described in standard NP EN ISO 9237:1997, applying a pressure of 100 Pa and using a 20 cm² test area. The equipment used was a Textest FX 3300 Air Permeability Tester.

Water vapour permeability

Water vapour permeability was determined according to the procedures described in BS 7209:1990. The equipment used was a Shirley instruments Water Vapour Permeability Tester.

Thermal properties

Thermal properties (thermal conductivity, thermal resistance and thermal absorptivity) were determined using Alambeta equipment.¹⁵

Microorganisms and culture media

Antibacterial properties were tested for gram-positive (*S. aureus* ATCC[®] 6538TM) and gram-negative (*E. coli* ATCC[®] 25922TM and *P. aeruginosa* ATCC[®] 27853TM) bacteria. Antifungal activity was tested against yeasts (*C. albicans* ATCC[®] 10231TM) and filamentous fungi/dermatophyte (*T. rubrum*, a clinical isolate from athlete's foot FF9).

Bacteria were cultivated in Mueller-Hinton Agar (MHA-Biomérieux), Tryptic Soy Agar (TSA-Liofilchem Diagnostic), Mac Conkey Agar (MAC-Liofilchem Diagnostic) for *E. coli* and *P. aeruginosa*, and Manitol Salt Agar (MSA-Liofilchem Diagnostic) for *S. aureus*. Sabouraud Dextrose Agar (SDA-Biomérieux) and CHROMagar Candida (Becton Dickinson) were used for *C. albicans* and Mycosel Agar (MYC-Becton Dickinson) for *T. rubrum*. Tryptic Soy Broth (TSB-Liofilchem Diagnostic) and Sabouraud Dextrose Broth (SDB-Liofilchem Diagnostic) were used as liquid media for bacteria and fungi, respectively.

Overnight cultures of bacteria and 24 hour cultures of yeast on MHA or SDA, respectively, were used to prepare a cell suspension in sterile 0.85% saline solution at the standard density of 0.5 Mc Farland. The inoculum contained microorganisms in the range of 1-1.5x10⁸ colony forming units (CFU) mL⁻¹. For dermatophytes, a spore suspension was prepared in 0.85% sterile saline solution with Tween 80, containing about 2.5-6 x 10⁵ CFU mL⁻¹, as counted in a Neubauer chamber.

Antibacterial and antifungal activity measurements

The antimicrobial activity of the knitted spacer fabrics (samples 1 to 8) and knitted flattened fabrics (samples 9 to 11), impregnated with thyme oil, was tested using selective media by agar diffusion methods, including a qualitative standard test – Parallel Streak Method AATCC TM147. Quantitative standard AATCC TM100 was used to confirm the biocidal or biostatic activity using *E. coli* and *S. aureus* as models.

Fabrics impregnated with thyme essential oil (samples 1-8, 10, 11) were tested by the qualitative agar diffusion method. Untreated sterilised swatches were used as negative controls in each experiment.

Antibacterial and antifungal qualitative evaluation

Three different approaches were used:

1) Fabrics (15 mm) were placed on the centre of plates containing the appropriate medium that was previously inoculated with each test microorganism and dried for 15 minutes.

2) Fabrics (15 mm) were submersed in suspensions of bacteria, yeast or fungal spores and incubated for 1 h at 37 °C. These samples were placed on agar plates.

3) The protocol proposed by standard method AATCC TM147 – Parallel Streak Method and standard method AATCC TM30.

The plates with bacteria were incubated for 24 h at 37 °C, with yeast – for 48 h at 37 °C, and with dermatophytes – for 5-7 days at 28 °C.

Inhibitory zones or absence of growth indicate antimicrobial activity. The inhibition was assessed from the zone formed under and around the fabric.

Antibacterial quantitative evaluation

The antibacterial properties of the fabrics were determined according to reference method AATCC TM100.

Circular swatches (4.8 cm in diameter) of each fabric and negative controls were sterilized before use. One mL of the 24 h culture broth of test bacteria (*S. aureus* and *E. coli*) in TSB medium, with about 3x10⁷ CFU mL⁻¹, was applied to the samples until complete absorption. Soon after inoculation (T0, contact time), 100 mL of 0.85% sterile saline solution was added to each inoculated test sample, inoculated negative control, and inoculated samples as sterilization control. After vigorous shaking, serial dilutions were prepared and 1 mL of each one was incorporated into agar medium. The other inoculated samples were incubated at 37 °C for 20 h. After the contact period, the viable bacteria were recovered according to the procedure described above. Agar plates were incubated at 37 °C for 48 h and the CFU was determined. The percent reduction (R%) of bacteria was calculated according to the equation $R (\%) = 100 (B-A)/B$, where A is the number of bacteria recovered after a 20 h contact period and B is the number of bacteria recovered immediately after inoculation.

RESULTS AND DISCUSSION

The 3D spacer textile structures were evaluated in relation to breathability and thermal comfort.

Air permeability values indicate ease of air flow through the fabrics and the main factors influencing this parameter are porosity, thickness,

mass per unit area, density, structure and the characteristics of the yarns.²² Some of the main properties of the textile structures developed are shown in Table 2. The results presented in Figure 1 show a correlation between porosity and air permeability. This behaviour can be expected as most porous materials with relatively open structures allow air to circulate more readily. Using 3D polyester warp knitted fabrics, He¹⁶ reported similar findings.

The ability of fabrics to transfer water vapour from the skin to the outer surface of the material is borne out by the values for water vapour permeability (Table 2). The highest values were obtained for polyester/bamboo at 30/70 (sample 5) and 61/39 (sample 6). The lowest values were noted for cotton and polyester combinations at 28/72 (sample 1) and 61/39 (sample 2). Our results show that the composition of the contact face is a key factor in determining fabric permeability to water vapour. Fabrics with polyester in the contact face and bamboo or cotton in the back faces showed the best results in terms of permeability to water vapour. Water vapour transmission takes place along the fibres and through the air spaces between the voids inside the structure. Accordingly, higher porosity facilitates the steam dissipation process through the material. Similar findings were reported by Rajan *et al.*¹³ on water vapour permeability using spacer fabrics made by mono- and multi-filaments of polyester.

Fabric water vapour permeability also depends on the physicochemical properties of the constituent fibres, as water vapour transfer also occurs through them. Therefore, water vapour permeability of the material depends on their moisture regain capacity and hygroscopic properties of the constituent fibres and generally increases with the degree of hygroscopicity. Figure 2 shows the results for air and water vapour permeability. It can be seen that the increase in air permeability is associated with an increase in fabrics water vapour permeability. Materials with the highest breathability were samples 5 and 6, both of which have 100% polyester in the contact side and 100% bamboo in the back side. The only difference between these samples is the yarn spacer.

The water vapour permeability values of all developed materials were higher than 2.0 mg.cm⁻².h⁻¹. These comply with ISO/TR 20882:2007(E), in which the performance requirements for lining

and insock components for footwear are established irrespective of the material used.

The properties related to the thermal comfort of materials, thermal conductivity, thermal resistance and thermal absorptivity were measured using Alambeta apparatus. For fibrous material structures with entrapped air, the thermal conductivity is the result of a combination of conduction and convection mechanisms, mediated by the fibre polymer and air entrapped in the porosity of the material. Therefore, it is more convenient to compare the apparent density of materials with thermal conductivity, as presented in Figure 3.

Materials with higher apparent density values showed higher thermal conductivity. Sample 2 showed better heat conduction than might be expected from the cotton content in the inner face and spacer. It is well known that polyester has higher thermal resistivity than cellulosic fibres. Moreover, 100% cotton has higher conductivity than 100% bamboo, 52.8x10⁻³ Wm⁻¹K⁻¹ against 43.2x10⁻³ Wm⁻¹K⁻¹, as reported for plain knitted fabrics.¹⁷

The thermal resistance behaviour was opposite to that of thermal conductivity, as might be expected (results not shown). The heat absorption capacity produces a heat-cool feeling on initial contact with the skin. High thermal absorption capacity imparts a sense of coolness on first contact. The heat-cool feeling is also determined by the roughness. Low roughness implies greater contact area and therefore greater heat transfer between the skin and the material. As the thermal absorptivity is directly related to the thermal properties of the material and the contact area, these two factors need to be considered. Clearly, sample 2 had higher absorptivity on both sides (Table 3). These values can be explained by the lower porosity of this sample compared to the others studied. This higher density material has higher real surface contact, and in this case, the driving energy transfer occurs faster, inducing greater cool feeling.

Extensive qualitative and quantitative tests were carried out to assess the antimicrobial properties of the 3D spacer fabrics and knitted flattened fabrics. No antibacterial or antifungal activity was observed for the knitted spacer fabrics (samples 1 to 8), using diffusion methods, including AATCC TM147. All the tested microorganisms grew in the respective media, exhibiting similar behaviour relative to the controls.

Table 2
Main properties of 3D textile structures

Sample	Weight (g.m^{-2})		Thickness (mm)		Apparent density (g.cm^{-3})	Porosity (%)	Air permeability ($\text{L.m}^{-2}.\text{s}^{-1}$)		Water vapour permeability ($\text{mg.cm}^{-2}.\text{h}^{-1}$)		Thermal conductivity ($\text{W.m}^{-1}.\text{K}^{-1}$) ($\times 10^{-3}$)	
	Mean value	CV (%)	Mean value	CV (%)			Mean value	CV (%)	Mean value	CV (%)	Mean value	CV (%)
1	476	1.2	3.29	2.9	0.145	89.9	1237	2.0	2.7	1.8	47.5	0.9
2	466	0.8	2.85	2.5	0.163	89.0	1174	3.4	2.8	1.2	52.2	1.4
3	294	1.6	2.60	2.8	0.113	91.7	2397	5.1	3.0	3.7	45.4	1.5
4	268	2.5	2.24	3.8	0.120	91.5	2425	3.4	3.1	3.3	43.4	2.7
5	273	1.9	2.24	4.6	0.122	90.9	2847	2.3	3.2	2.2	41.5	0.9
6	260	2.3	2.37	4.8	0.110	91.9	2984	3.5	3.3	3.5	41.3	0.6
7	297	1.5	2.49	4.2	0.119	91.7	2019	3.8	3.0	3.6	45.5	1.1
8	324	2.6	2.54	3.9	0.128	90.9	2209	1.8	3.1	2.9	44.7	1.3

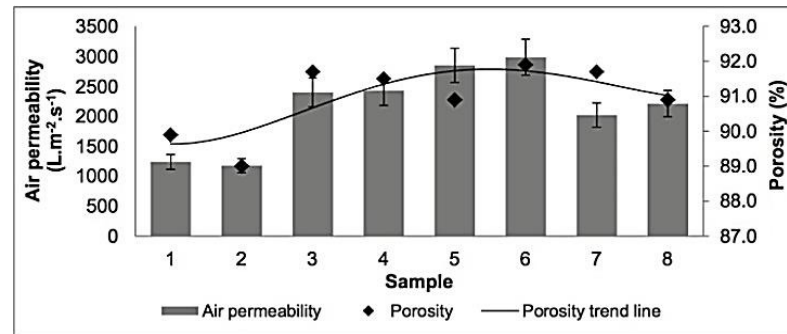


Figure 1: Air permeability and porosity of 3D spacer textiles

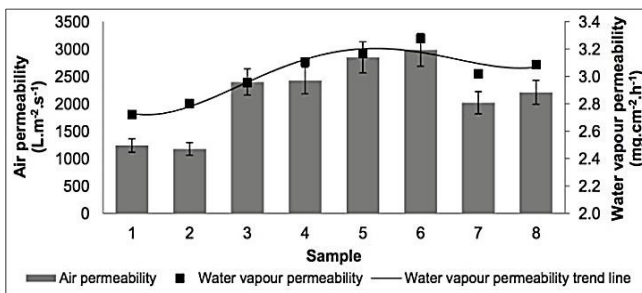


Figure 2: Air and water vapour permeability of 3D spacer textiles

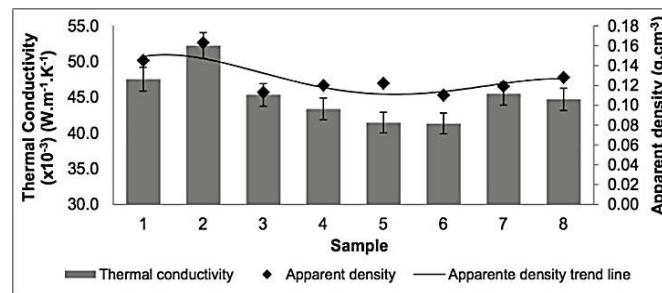


Figure 3: Effect of apparent density on thermal conductivity of 3D spacer textiles

Table 3
Thermal absorptivity of 3D spacer textiles

Sample	Porosity (%)	Composition (Face A/Face B)	Thermal absorptivity (W.s ^{1/2} .m ⁻² .K ⁻¹)	
			Mean value	CV (%)
1	89.9	Bioactive polyester	85.02	2.4
		Cotton	86.44	3.1
2	89.0	Bioactive polyester	102.78	2.7
		Cotton	94.96	2.2
3	91.7	Polyester/Cotton	78.2	4.5
		Bamboo	90.74	3.9
4	91.5	Bamboo	90.56	3.4
		Polyester	75.08	3.2
5	90.9	Bamboo	84.28	2.8
		Polyester	73.32	2.3
6	91.9	Bamboo	73.62	3.8
		Polyester	67.12	2.9
7	91.7	Bioactive polyester	78.6	4.1
		Cotton	77.42	3.3
8	90.9	Bioactive polyester	74.94	4.1
		Cotton	78.54	3.7

The bamboo-based material (sample 9) was the only sample showing activity against gram-negative bacteria, *E. coli* and *P. aeruginosa*, and the dermatophyte, using diffusion methods. The antimicrobial profile of the samples was also tested using the AATCC TM147 protocol and gave similar results. However, the gram-positive bacteria *S. aureus* and yeast *C. albicans* were not affected, suggesting some selectivity of this material.

The tested knitted flattened bamboo based fabrics (100%) with porosity of 81.70% retained antimicrobial activity against *E. coli*, *P. aeruginosa* and *T. rubrum*. The last is particularly important, considering its involvement in dermatophytosis. When applying the diffusion methods, the contact between the material and the microorganism could be incomplete and different antimicrobial agents diffuse through agar at different rates or not at all. To verify the antibacterial efficacy and the effect of the direct contact of the microorganisms with the samples, the AATCC TM100 quantitative method was used for gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria. This test analyses the antimicrobial efficacy of the material after direct contact with the microorganisms over a determined time period. In contrast to AATCC TM147 and other agar diffusion tests, it is independent from the diffusion properties of the antimicrobial agent. Moreover, it allows a quantitative evaluation of antimicrobial activity and the results are retrieved as percentage reduction of microbial counts. For samples 1 to 8 and negative control (cotton), the number of viable cells duplicated from 0 h (10^4 - 10^5 CFU) to 20 h of contact (10^8 - 10^9 CFU). No bacteriostatic or bactericidal effect was observed for the knitted spacer fabrics against *S. aureus* and *E. coli*, which showed a performance similar to that of the control sample. The results confirm that the lack of activity in the qualitative test is independent of the diffusion and contact.

Bamboo fabric (sample 9) reduced the number of viable cells of *S. aureus* after 20 h of contact, but this effect was not seen using the diffusion method. The inhibitory activity previously described for *E. coli* and revealed by the AATCC TM147 test was confirmed by the AATCC TM100 method. *E. coli* and *S. aureus* not only proved to be unable to multiply in the presence of the bamboo fabric, but also the number of initial cells was strongly reduced by 50 to 96%. As in

AATCC TM147, no inhibitory effect was observed for the remaining samples.

On the other hand, the antimicrobial efficiency of the fabric impregnated with thyme essential oil depends directly on the concentration. Comparing the MIC determined for the different microorganisms to *T. vulgaris*, according to CLSI methods, the most resistant was *P. aeruginosa* (MIC 2.5-5 $\mu\text{L mL}^{-1}$) and the most susceptible was *T. rubrum* (MIC 0.04 $\mu\text{L mL}^{-1}$). Moreover, *S. aureus* and *E. coli* showed a MIC of 0.16 $\mu\text{L mL}^{-1}$, whereas the MIC for *C. albicans* was 0.32 $\mu\text{L mL}^{-1}$. The highest resistance of *P. aeruginosa* to thyme oils was also described to *T. pulegioides*³¹ and to *T. vulgaris*.³² The differences between the gram-positive and gram-negative bacteria may be related to the different ultrastructure of the cell wall. However, *E. coli* and *P. aeruginosa* are both gram-negative and the oil is much more active against *E. coli*, similarly to *S. aureus* (gram-positive), than against *P. aeruginosa*. Our results confirm the higher resistance of *P. aeruginosa* to antimicrobial compounds.

However, only fabrics impregnated with 2.5 $\mu\text{L mL}^{-1}$ (0.25%) of thyme essential oil showed activity against all the microorganisms tested. *Pseudomonas aeruginosa* was the exception, requiring the application of 5 to 10 $\mu\text{L mL}^{-1}$ (0.5-1%) of the oil in fabrics, which is higher than its MIC value. This need for a higher concentration than the determined MIC could be due to partial evaporation of volatile components of the oil during the drying process.

CONCLUSION

The knitted spacer fabric produced with natural yarns of bamboo and cotton combined with polyester showed good breathability, compatible with the use as shoe lining material. The air permeability values for this material were above 2000 Lm^2s^{-1} and permeability to water vapour complied with the standard specified by ISO/TR 20882:2007(E).

The properties relating to thermal comfort revealed that these materials are suitable for use in cold weather due to their low thermal conductivity. Out of the different combinations of materials studied, the bamboo/polyester material was the best in terms of comfort, assuming that the hydrophobic face is in contact with the skin. The strategy of using inherently antimicrobial fibres has not been successful to produce antimicrobial fabrics.

It seems that the porosity of the 3D spacer samples implies insufficient contact surface area to produce inhibition of the tested microorganisms. Thus, we realized the need to add a leaching antimicrobial that freed up to the middle. *Thymus vulgaris* oil was tested as a model antimicrobial additive due to the natural and biocompatible behaviour and antimicrobial properties, especially the antidermatophytic one. The samples impregnated with oil showed antimicrobial activity against *E. coli*, *S. aureus*, *C. albicans* and *T. rubrum* and could be thus considered as an alternative material suitable for footwear lining to prevent athlete's foot and related bacterial infections. Bamboo also showed activity against *T. rubrum*, which can be important to avoid mycoses. Also, activity of bamboo against bacteria could help in co-infections. The knitted spacer fabrics with antimicrobial performance are suitable for applications where washing fastness is not a critical requirement.

In conclusion, the 3D spacer materials based on natural bamboo/polyester with thyme essential oil as antimicrobial agent can be a suitable alternative to the fully synthetic materials usually used for shoe lining, combining comfort and antimicrobial properties.

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REFERENCES

- ¹ M. Ghannoum and N. Isham, *PLOS Pathog.*, **10**, e1004105 (2014).
- ² H. Sanada, G. Nakagami, K. Takehara, T. Goto, N. Ishii *et al.*, *Healthcare*, **2**, 183 (2014).
- ³ J. Yip, L. Liu, K. H. Wong, P. H. M. Leung, C. W. M. Yuen *et al.*, *J. Appl. Polym. Sci.*, **131**, 8886 (2014).
- ⁴ M. Negri, T. P. Salci, C. S. Shinobu-Mesquita, I. R.

- G. Capoci, T. I. E. Svidzinski *et al.*, *Molecules*, **19**, 2925 (2014).
- ⁵ M. Shahid and F. Mohammad, *Ind. Eng. Chem. Res.*, **52**, 5245 (2013).
- ⁶ B. Simoncic and B. Tomsic, *Text. Res. J.*, **80**, 1721 (2010).
- ⁷ M. M. G. Fouda, E. S. Abdel-Halim and S. S. Al-Deyab, *Carbohydr. Polym.*, **92**, 943 (2013).
- ⁸ R. A. Monticello and P. D. Askew, in “Russell, Hugo & Ayliffe’s: Principles and Practice of Disinfection, Preservation and Sterilization”, Wiley-Blackwell, Oxford, UK, 2013, pp. 520-529.
- ⁹ E. Pinho, M. Grootveld, G. Soares and M. Henriques, *Carbohydr. Polym.*, **101**, 121 (2014).
- ¹⁰ S. C. Davis and R. Perez, *Clin. Dermatol.*, **27**, 502 (2009)
- ¹¹ H. Haufe, K. Muschter, J. Siegert and H. Böttcher, *J. Sol-Gel Sci. Technol.*, **45**, 97 (2007).
- ¹² M. M. Cowan, *Clin. Microbiol. Rev.*, **12**, 564 (1999).
- ¹³ C. Pina-Vaz, A. Gonçalves Rodrigues, E. Pinto, S. Costa-de-Oliveira, C. Tavares *et al.*, *J. Eur. Acad. Dermatol. Venereol.*, **18**, 73 (2004).
- ¹⁴ E. Pinto, C. Pina-Vaz, L. Salgueiro, M. J. Gonçalves, S. Costa-de-Oliveira *et al.*, *J. Med. Microbiol.*, **55**, 1367 (2006).
- ¹⁵ L. R. Salgueiro, E. Pinto, M. J. Gonçalves, C. Pina-Vaz, C. Cavaleiro *et al.*, *Planta Med.*, **70**, 572 (2004).
- ¹⁶ P. Mollarafie, P. K. Parsi, R. Zarghami, M. A. Fazl and R Ghafarzadegan, *J. Med. Plants*, **14**, 69 (2015).
- ¹⁷ Y. Chang, L. McLandsborough and D. J. McClements, *J. Agric. Food Chem.*, **60**, 12056 (2012).
- ¹⁸ D. De Smet, M. Vanneste, K. Leppchen-Fröhlich and M. Meyer, *Melliand Int.*, **1**, 45 (2015).
- ¹⁹ C. Ringot, V. Sol, R. Granet and P. Krausz, *Mater Lett.*, **63**, 1889 (2009).
- ²⁰ L. Windler, M. Height and B. Nowack, *Environ. Int.*, **53**, 62 (2013).
- ²¹ E. Pinho, M. Henriques, R. Oliveira, A. Dias and G. Soares, *Fibers Polym.*, **11**, 271 (2010).
- ²² P. Taylor, K. M. Babu and K. B. Ravindra, *J. Text. Inst.*, **1**, 37 (2015).
- ²³ T. P. Rajan, L. D. Souza, G. Ramakrishnan and G. M. Zakriya, *J. Ind. Text.*, **11**, 1 (2014).
- ²⁴ M. Blaga, A. R. Ciobanu, A. Marmarali, G. Ertekin and P. Çelîk, *Tekstil ve Konfeksiyon*, **25**, 111 (2015).
- ²⁵ E. Ghorbani, M. Zarrebini, H. Hasani and M. Shanbeh, *J. Text. Light Ind. Sci. Technol.*, **4**, 17 (2015).
- ²⁶ L. Hes, M. De Araujo and V. V. Djula, *Text. Res. J.*, **66**, 245 (1996).
- ²⁷ T. He, Master Thesis, North Carolina State University, USA, 2011.
- ²⁸ A. Majumdar, S. Mukhopadhyay and R. Yadav, *Int. J. Therm. Sci.*, **49**, 2042 (2010).
- ²⁹ I. Boz, E. Gille, R. Necula, S. Dunca and M.-M. Zamfirache, *Cellulose Chem. Technol.*, **49**, 169 (2015).
- ³⁰ O. Borugă, C. Jianu, C. Mișcă, I. Golet, A. T. Gruiă *et al.*, *J. Med. Life*, **7**, 56 (2014).