(REFEREED RESEARCH)

ECO-FRIENDLY ANTIBACTERIAL FINISH FOR NATURAL KNITTED FABRICS

DOĞAL LİFLERDEN ÖRÜLMÜŞ KUMAŞLARA ÇEVRE DOSTU ANTİBAKTERİYEL BİTİM İŞLEMİ

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ABSTRACT

The present research is focused on the fabrication and characterization of eight knitted fabrics loaded with beeswax/sage essential oil system by a two-step process: oil-in water (o/w) emulsification, and application on knitted fabric. The investigation was designed to determine antibacterial effect, the ability to release the biologically active compound, and comfort indices (air and vapor permeability and hygroscopicity) of treated knitted fabrics. We have clarified the successful entrapment of sage essential oil (SEO) in beeswax matrix by UV-VisSpectro-photometry and have determined the morphology of treated knitted fabrics by Optical Microscopy.

Keywords: Sage essential oil; Beeswax; Emulsion; Staphylococcus aureus; Knitted fabric.

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1. INTRODUCTION

Textile materials with antibacterial properties are of a special interest because they can act as a barrier against microbial contamination [1]. Among these, with great interest were welcome the knitted fabrics made of natural fibers, which have different medical applications due to their advantages: soft touch, air permeability, bio-degradability, high surface area, absorbency phenomenon, and variety in product forms [2-6]. Among the natural fibers, the flax ones are the most resistant to microbial attack. Flax materials are used in medicine as support for patches and bandages, due to their capacity to maintain the optimum humidity and to restrain the microorganism contaminated area [7-8].

For preventing the wound from drying out, textile support must be coated with an active system which will provide the necessary humidity [9]. Generally, woven fabrics have bioactive agents that are physically absorbed or adsorbed; theyarecoated, encapsulated or covalently bound to the fabric. Among bioactive agents, a special interest presents the essential oils, due to biological active principles which they include in their composition [10-12]. Most of times, the major essential oil component may not be the only one responsible for the antimicrobial activity but a synergistic effect may take place with other oil components [13]. The application of essential oils on industrial scale is limited due to their volatility. Encapsulation of essential oil diminishes their volatility and controls their release in time [14]. A successful micro-encapsulation must retain maximum active material. The choice ofcapsulecoating material influences both the emulsion properties, and the characteristics of the formed particles. Some biopolymers used as coating material for active compound encapsulation include gum Arabic, maltodextrin, modified starches, whey protein concentrate, mesquite gum and chitosan [15-18]. The sage essential oil is used quite often due to its antioxidant, anti-inflammatory, antimicrobial and antifungal properties [19-22]. Among these, 1,8-cineol has the highest antimicrobial activity [23-26].

The objective of the present work was to develop and characterize some materials with antibacterial properties, obtained by applying the beeswax/sage essential oil system on different types of knitted textile supports. Beeswax is an inactive ingredient used to produce stable emulsions of even consistency and to help ensure a controlled release of the active compound (sage essential oil), as well as stability and safety. A possible destination for antimicrobial knitting fabrics with controlled release of essential oil could be therapeutic armrests.

2. MATERIALS AND METHODS

2.1.Materials

The following substances were used for emulsion preparation: Beeswax (procured from a private apiary in the North-East region of Romania), Chitosan (molecular weight 100.000 - 300.000 andde-acetylating degree 85%, obtained from FlukaChemie GmbH – Switzerland); Sage essential oil-extract of *Salvia Officinalis* L, was purchased from Fares SA Romania; Emulsifier (Tween 80), supplied from Merck – Germany; 99.5% purity Vegetable glycerinpurchased from Elemental company, Romania.

The investigations were carried out with knitted samples made from several types of yarns on two types of knitting machines, as presented in Table 1.

Table 1. Characteristics of yarns and fabrics

Variant	Fiber type	Linear density (Tex)	Knitted structure	Type of knitting machine
V.1.	Flax	25	single jersey	circular knitting machine
V.2.	Flax	50	rib	flat knitting machine
V.3.1.	Flax	40	single jersey	flat knitting machine
V.3.2.	Flax	40	rib	flat knitting machine
V.4.1.	Flax	50	single jersey	flat knitting machine
V.4.2.	Flax	50	rib	flat knitting machine
V.12.1.	Cotton/regenerated bamboo (50/50)	28	single jersey	flat knitting machine
V.12.2.	100% eco- friendly cotton	25	single jersey	circular knitting machine

The knitted fabrics were produced in single jersey and 1:1 rib structures. Part of the single jersey samples were knitted on a small diameter circular knitting machine “Lab-Knitter” 294E (from Mesdan-Lab) with 3¾ inches diameter and one knitting system. The rest of single jersey and 1:1 rib samples were produced on a 12E manually operated flat knitting machine.

2.2. Analysis of structural parameters of knitted fabrics

For this research, the most significant knitted fabric parameters were measured. Number of courses per centimeter (CPC) and number of rows per centimeter (RPC) were measured at ten different places on every sample using a magnifying glass, and then the average values and the stitch density per square centimeter were calculated. The thickness was measured using Digital Thickness Gauge equipment (M034A) and the average of ten measured values for each sample (belonging to each variant) was calculated. The loop length (*l*) was calculated by dividing the average of unraveled length of yarn by the number of stitches (20). Tightness factor (*TF*) was calculated using formula 1.

$$TF = (Tex)^{1/2}/l \quad (1)$$

2.3. Production and application of beeswax/sage essential oil on textile support

Beeswax in the concentration range of 1.4%÷12.5% was melt at about 63^0C in a thermo regulated water bath. Beeswax was added to the water phase heated at a temperature increasing by 5°C up to above the melting point of wax, allowing sufficient time to equalize the temperatures of both phases. Emulsifier (Tween 80) was added to the chitosan/beeswax mixture. Glycerin solution was added to the heated mixture which was stirredfor10 minutes. After complete homogenization,sage essential oil was added drop-wise to the continuously stirred mixture. The eight knitted fabric variants were treated with the emulsion obtained according to the formula presented in Table 2.

Table 2. Chemical composition of emulsion

Components	Composition(% w/v)
Wax	4.10
Sage essential oil	26.67
Glycerin	14.10
Tween 80	14.10
Water	41.03

The knitted fabrics (3 g) were padded with mixtures to a wet pick-up of 100%, and dried.

2.4. Emulsion characterization

Microscopic evaluation of solutions

Microscopic evaluation of hydro-glycerinated emulsions obtained according to the eight treatment variants was performed by using an optical microscope (KRÜSS), and the microscopic images were transferred for computerized analysis using a digital camera (Nikon, Coolpix P 5100).

Turbidity determination

Emulsion turbidity obtained according to Table 2 was calculated according to relation 2:

$$\tau = -\frac{\ln\left(\frac{\%T}{100}\right)}{L}, (cm^{-1}) \quad 2$$

where:

τ- turbidity index; *T*- transmittance read on spectrophotometer; *L*- length of the side of spectrophotometer vat, cm.

2.5. Profile of biological active compound release

In order to determine the cumulative quantities of essential oil released in time, a known quantity of treated knitted

fabric was immersed in 50 ml normal saline solution and incubated at the temperature of 37°C under slow and constant stirring (40 rpm). At established time intervals, the solutions were filtered and subjected to spectrophotometer analysis using aCampsec M501 Single Beam Scanning UV/Visible Spectrophotometer. Measurements were repeated three times at room temperature, using 2 mm quartz cells.

2.6. Antimicrobial analysis

The antimicrobial activity of the suspensions created with essential oils was tested through the Kirby-Bauer method standardized by the Clinical and Laboratory Standards Institute (CLSI). This method is frequently used for antimicrobial testing from medical area and adapted to test other types of materials too. The antimicrobial activity was tested against various standardized Gram-positive and Gram-negative bacterial suspensions.Evaluation of sensitivity degree consists in the appearance and determination of inhibition zone around the tested sample [27]. Many bacteria are able to develop changes in their sensitivity, but *Staphylococcus aureus* and *Escherichia coli* have been recognized for the increasing resistance to conventional antibiotics [28-31].Antimicrobial testing was performed on standardized *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 stems, both being recommended by CLSI for agar diffusion method, from the collection of Microbiology Laboratory, Public Health Department from the Faculty of Veterinary Medicine of Iassy, Romania.For this, we have obtainedyoung cultures of 24 h, in liquid growth medium (nutritive bouillon). The bacterial inoculus was brought at the standard density of 0.5 Mc Farland, at which1 ml of suspension contains 1÷2 x 10^8cfu/ml (colonies forming units) for most of the bacterial species.

Muller Hinton agar (Oxoid) was used as solid nutritive medium, which possesses a nutritive value that permits the optimum development of a wide variety of germs and does not contain inhibitors of the action of some antimicrobial substances. After melting and cooling down to 45°C, quantities of 9mL were distributed on sterile Petri plates, on which 1 mLof the tested bacterial culture from the dilution tube was deposed.Disc-shaped samples with the diameter of 1.5 cm were taken from the textile materials treated with various essential oils (similar to the diffusimetry method with discs).

The samples were placed circularly at equal distances, on the surface of a solidified Muller Hinton medium. The plates were incubated in thermostat at 37°C, the results being interpreted after 24 h. The assessment of antimicrobial effect consisted in measuring the diameter of the inhibition zone created around the textile material disc. This is directly proportional with the sensitivity of the reference bacterial stem such that, the more active are the substances from the essential oils, the more extended is the inhibition zone (within which bacterial colonies are not developing).

2.7. Analysis of comfort indices

The analysis of comfort indices was based on the air permeability, water vapor permeability and hygroscopicity which were determined for the studied samples. The air permeability was determined according to SR EN ISI 9237 Standard on METEFEM apparatus (Hungary) using a pressure difference of 10 mm water column. Water vapour permeability, was evaluated on Permetest instrument (Czech Republic) according to ISO 11092:1993 Standard. Hygroscopicity was determined according to the EN ISO 12571:2000 Standard.

2.8. Statistical analysis

The experimental data concerning turbidity, controlled release, antibacterial activity and comfort indices were expressed as the mean value ± standard deviations. Reliability limits were set at $p< 0.05$. Standard deviations did not exceed 5% for the majority of the obtained values.

3. RESULTS AND DISCUSSIONS

3.1. Knitted fabrics analysis

Images and graphical representations for two of the variants of the knitted samples used for investigations are presented in Figure 1 and the selected structural parameters for all samples are included in Table 3.

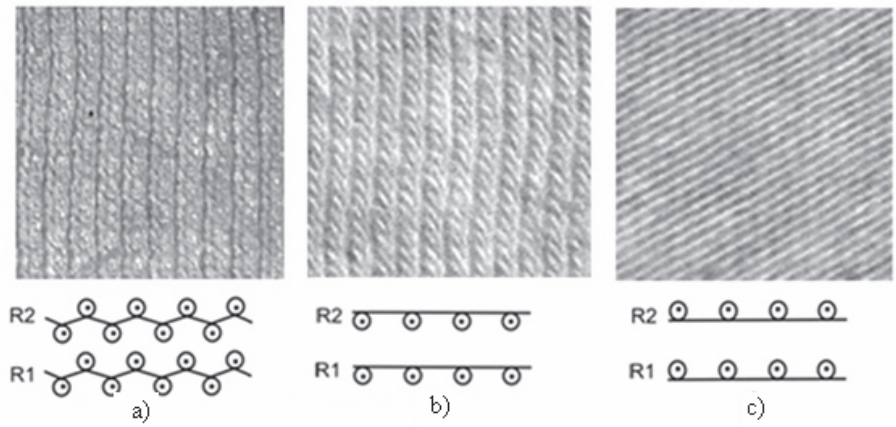

Figure 1. Images and graphical representations of the knitted samples: a) variant V.2 - rib; b) Variant V.4.1-single jersey (front); c) Variant 4.1 – single jersey (back)

Table 3. Structural parameters of the knitted fabrics

Treating variants	Stitch density	Thickness, mm	Tightness Factor (TF)
V.1.	124.00	1.12	1.55
V.2.	35.360	1.92	0.90
V.3.1.	53.760	1.30	1.33
V.3.2.	43.520	2.03	1.18
V.4.1.	53.760	1.39	1.54
V.4.2.	33.440	2.20	1.38
V.12.1.	227.04	0.88	1.55
V.12.2.	245.00	0.85	1.67

After knitting, all the samples were scoured for paraffin removal and dry relaxed by keeping them on a flat surface in the laboratory atmospheric conditions for at least 48 hours in order to release the strains.

3.2. Characterization of the obtained emulsions and knitted fabrics

The time stability of some emulsions was studied by reading the extinction and transmittance respectively, on Campsec M501 Single Beam Scanning UV/Visible Spectrophotometer in order to determine the turbidity, pursuing its modification at maximum wavelength, λ_{max}. Turbidity was calculated with the relation (1). With this end in view, about 0.5 ml diluted emulsion sample (1:600, v/v) was introduced in the spectrophotometer cell with lid, with the length of spectrophotometer cell side of 1 cm, being afterwards deposed at room temperature for 24 h. The characteristics of the obtained emulsion are presented in Figs. 2 and 3.

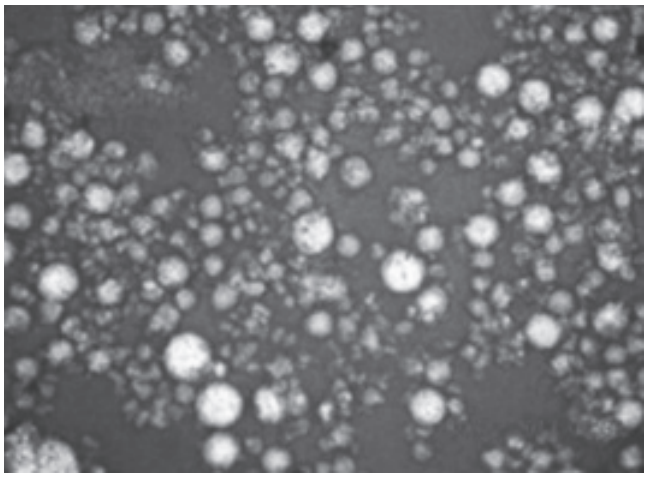

Figure 2. Micrograph of the emulsion

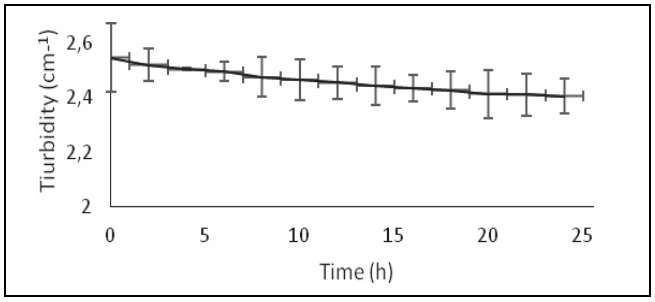

Figure 3. Emulsion turbidity

From the analysis of time variation of the emulsion turbulence, one can notice a slight decrease of its stability during the 24 hours since emulsion formation. What concerns the microscopic aspect of the obtained emulsion, one can notice a uniform distribution of the particle size. The application of the emulsion on the surface of the eight knitted fabrics variants did not result in significant modifications of their morphological aspect. The obtained results are presented in Figure 4.

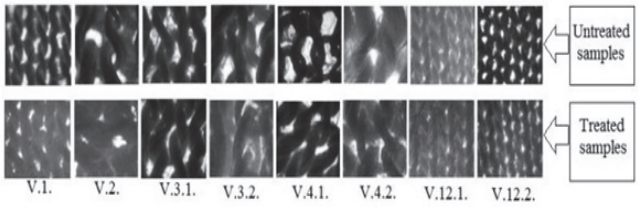

Figure 4. Images of knitted fabrics untreated and treated with emulsion respectively

3.3. Study of the capacity to release the biological active compound

The concentration of sage essential oil (SEO) released at the moment *t* was calculated from the equation of the calibrated straight line $y = 0.3034x$, where *x* represents the concentration of the solution of biologic active substance, and *y* is the absorbance given by UV spectral analyses. The quantity of essential oil released between 1h and 4 h was calculated from the data supplied by the standard curve.

The profiles of sage essential oil release are presented in Figure 5.

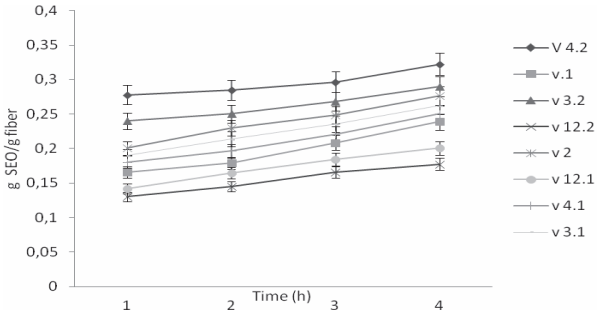

Figure 5. Profile of sage essential oil release

The release of the biologic active compound depends on structural parameters of the studied knitted fabrics. From the analysis of the profile of sage essential oil release from the eight knitted fabric variants, it follows that the treated fabrics produced in rib have the highest release. The release of the active compound also depends on the stitch density and thickness. Namely, the amount of sage essential oil released in time is directly proportional with the stitch density and inversely proportional with thickness.

3.4. Antimicrobial testing

The results of antimicrobial testing are presented in Figures 6 and 7.

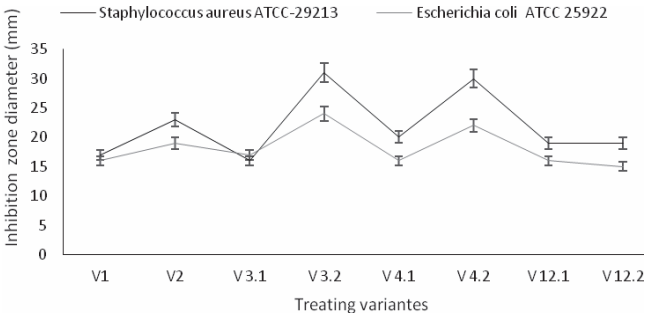

Figure 6. Diameter of inhibition zones for treated samples

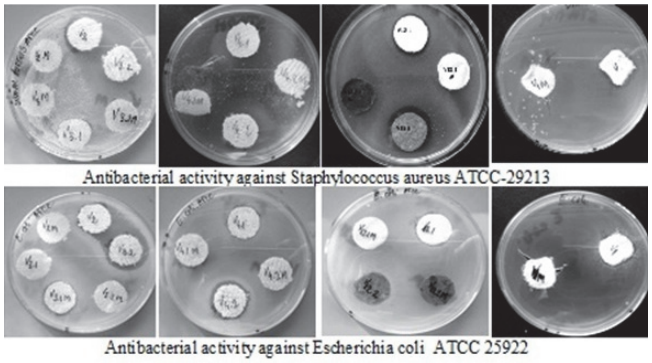

Figure 7. Antimicrobial activity of the samples

From the analysis of the obtained data one can notice that the Gram positive bacteria (*Staphylococcus aureus*ATCC-29213) are more sensitive to the active principles than the Gram-negative bacteria (*Escherichia coli* ATCC 25922). The best inhibiting effect on both *Staphylococcus aureus* and *Escherichia coli* was found at the samples V2, V3.2 and V4.2. The explanation consists in the different knitted fabric structure. Thus, the knitted fabrics produced in 1:1 rib presenta higher antibacterial effect, as compared to the knitted fabrics produced in single jersey (V.1, V.3.1., V.4.1, V.12.1, and V.12.2).

3.5. Analysis of the comfort indices

The comfort indices for the treated samples are influenced by the structural parameters of the knitted fabrics and by emulsion composition. Hygroscopicity values are higher for the treated samples than for the untreated sample, due to glycerin from emulsion composition (Figure 8).

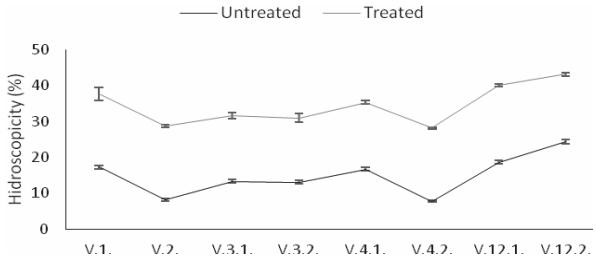

Figure 8. Hygroscopicity of the samples

What concerns the influence of knitted fabric structure, the highest hygroscopicity values were obtained for the densest samples, and for the samples with the highest loading degree, implicitly with the highest glycerin content. What concerns the vapor permeability, the samples with the highest stitch density has the smallest vapor permeability for both the treated and untreated samples (Figure 9). The treated samples present smaller vapor permeability than the untreated samples, due to their higher hygroscopicity.

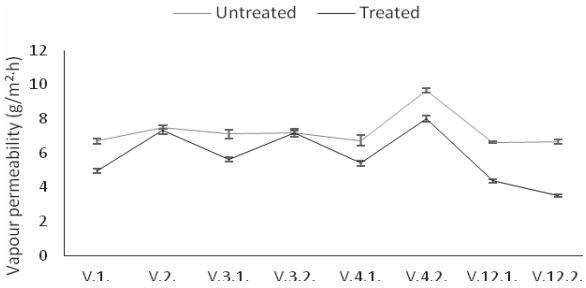

Figure 9. Vapor permeability of the samples

Air permeability decreases with the stitch density of the knitted fabrics, the permeability values being smaller for the treated samples than for the untreated samples. A possible explanation would be the deposition of matrix/active compound system on the yarn surface (Figure 10).

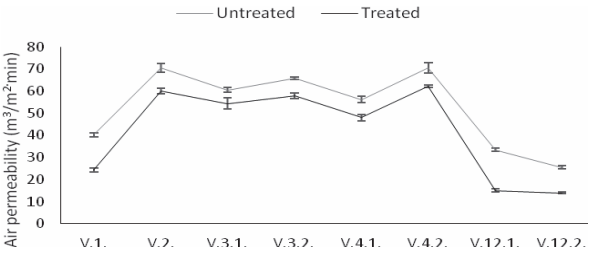

Figure 10. Air permeability of the samples

4. CONCLUSIONS

The present research was focused on the developing and characterizing of some materials with antibacterial properties, obtained by applying the beeswax/sage essential oil system on different types of knitted textile supports.

Emulsion composition ensures its high stability and does not significantly alter morphological appearance of the eight knitted samples analyzed. The data resulted from this research have proved the antimicrobial effect and the controlled release of the sage essential oil from the eight types of knitted fabrics made of natural fibers. Another important aspect of the research is given by the inter-dependence of the obtained effect and structural parameters of the knitted fibers. The knitted fabrics with small stitch density and big thickness present a better release of the sage essential oil and, accordingly, a better antimicrobial effect. Therefore, the nature of the knitted fabric influences the wanted result and its choice must be correlated with the final destination of the product. What concerns the comfort index, one can appreciate that hygroscopicity values are higher for the treated samples than for the untreated sample, and air and vapor permeability is lower at the treated samples.

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