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Lavender Essential Oil as Antibacterial Treatment for Packaging Paper

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Abstract: Based on many years of experience, packaging is considered as an inactive barrier that protects materials and goods from environmental factors. The applicability of native chemical additive such as essential oils in wrapping papers can be used in the forms of films, treatments, coatings or others. Essential oils or extracts from different aromatic plants are used as bioactive substances for antimicrobial activity. In this research, lavender essential oil treatment of packaging papers is examined for its inhibition activity under nine microorganisms—two Gram-positive bacteria, three Gram-negative bacteria, two yeast and two fungal strains. The effectiveness of the treatment on the structural and strength indicators of the obtained paper samples is monitored. In detail, a five-day examination is conducted on the antibacterial effectiveness of lavender essential oil treatment. Results indicate that the lavender treatment of the obtained packaging paper is successful and the antifungal effect is more pronounced. The antimicrobial efficiency of paper treated with lavender essential oil is between 60 and 90% in the first two hours after treatment and gradually decreases to 40%–50% at the end of the 120 h period. The lavender essential oil treatment of wrapping paper has a promising perspective for preserving products from microbial spoilage and extending their shelf life.

Keywords: antibacterial; lavender oil; paper; treatment; packaging

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1. Introduction

Essential oils (EO) are extracted from different parts of the aromatic herbs. They are volatile liquids and are defined based on their aroma conjunctions and ability to avert the growth of nourishing pathogens. These bioactive compounds are suitable for active packaging and preservation of food products. As essential oils contain a high number of bioactive compounds, they improve the antibacterial properties of the packaging material [1].

Undoubtedly, based on many years of experience, packaging is considered as an inactive barrier that protects materials and goods from environmental features such as pressure and heat, ultraviolet light, water, and oxygen. The advanced research proves that it could extend the shelf life of food products by protecting them from microbiological and chemical pollutants and providing the opportunity for daily goods to be safely transported and stored. In recent years, efforts have been made to implement innovations in the creation of packaging materials—modified atmosphere packaging, new active packaging and nanomaterials in different applications [2–6].

Active packaging, apart from its main purpose–protection, affects the storage of the packaged product. Through the newly developing technological approaches, it is becoming a promising tool for increasing the usage of biodegradable, sustainable and natural materials and products.

The applicability of native chemical additives such as essential oils in wrapping papers can be used in the forms of treatments, films, coatings or others. The literature review presents many reports of polysaccharides-based films that are efficient against

fungus and bacteria which spoil the food. One of the newest research trends in the food packaging industry is the use of herb natural extracts and oils in the composition of edible biopolymers [7–12].

Various essential oils are obtained from crops that are mainly harvested for this purpose (cinnamon, lavender, basil, thyme, rosemary, etc.), while other extracts or oils are gained as by-products processing of agricultural wastes (orange peel, grape seeds, apricot kernels, etc.). The main challenge in such type of everyday food and goods packaging is that the manufacturer has to match the type of the extract or oil with the type of the protected product. While the consumers of the meat-based products could easily use the essential oil of rosemary, thyme or ginger, not many of them would like them with vegetables or fruits. It could be expected about vanilla and cinnamon, which are enjoyable with pastry, contrary to meat. However, the essential oils of tangerine, lime or orange could be used as wrapping materials for seafood and fruits [13].

Some important disadvantages and limitations to the use of essential extracts and oils as active additives is their strong aroma, slight solubility and high-rise volatility, and the possibility of negatively influencing organoleptic properties of the packed product. Furthermore, extracts and oils are characterized with heat and light sensitivity, increasing the possibility of losing the active substances from the package. Prevention of this process is possible by encapsulation or nanoemulsification.

The literature review on the packaging and wrapping papers treatment with plant extracts reports antimicrobial analysis of extracts and oils from grapefruit, grape, pomegranate, cinnamon, horseradish and clove seeds [3,14]. Essential oils are widely used in aromatherapy, cosmetics, medicine and food industry. Depending on essential oils' composition, they exhibit different properties: antibacterial, antiviral, antifungal, antioxidant, antiallergic. Due to their preservative properties, they are important for food safety and preservation. Such are essential oils of oregano, cumin, thyme, basil, mint, sage, clove, eucalyptus.

Lavender oil has been used to extend the quality and shelf life of bread [15] as well as in strawberry storage packaging [16]. Lavender is a popular essential oil raw material from which quality and widely used aromatic products are obtained. Essential oil can be obtained from different types of lavender, but the highest quality is that of narrow-leaved lavender (Lavandula angustifolia Mill.). It has a high content of linalyl acetate and linalool [17–20]. In addition to antimicrobial activity, lavender oil also has sedative, carminative, antidepressant and anti-inflammatory effects [21]. It has also been determined that lavender oil can increase the antimicrobial activity of other compounds (hydroxyapatite nanoparticles) and thus develop new means to fight antibiotic resistance [12]. Synergism with antibiotics and antiseptics has been established. Lavender oil increases the activity of antiseptic agents such as octenidine dihydrochloride against methicillin-resistant strains of S. aureus [22]. The same effect was also demonstrated with a combination of lavender oil with four antimicrobial agents (nystatin, chloramphenicol, ciprofloxacin and fusidic acid) [23].

A recent approach benefiting the main active and antibacterial properties of the essential oils is the development of multicomponent films or composites with essential oils used in packaging application [24]. Therefore, the essential oils interfusion is increasingly frequent, based on their increased antioxidant and antibacterial efficiency, together with water vapor permeability reduction. Most of the published investigations on the EO activity are based on the Gram-positive and Gram-negative bacteria and few on other microorganisms. Scientific research reports on the treatment of wrapping paper with lavender oil are also rare. One of the new and interesting technologies is the application of encapsulating lavender essential oil in gelatin/gum-arabic complex coacervate and varnish screen-printing in making fragrant gift-wrapping paper [25].

Therefore, the objective of this research is to investigate the potential use of lavender EO treatment in wrapping paper production and to determine the structural and strength properties of obtained treated paper and its antibacterial activity over nine different bacterial fungal and yeast strains at 120 h of analysis.

2. Materials and Methods

2.1. Lavender EO

A manufacturer from Bulgaria provided the EO of lavender. The EO had been extracted from the flower of *Lavandula angustifolia* Mill. and purified by steam distillation.

The lavender EO's physical and chemical characteristics including its color, appearance, odor, and polarization co-efficient [20,26], as well as its refractive index (ISO 280:1998), relative density at 20 °C (ISO 279:1998), and acid number (ISO 1242:1999) were determined.

2.1.1. GC–MS Analysis

The lavender EO was analyzed by gas chromatography–mass spectrometry (GC–MS) as described in our previous work [27]. The GC–MS analysis was carried out on an Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass-selective detector, helium as carrier gas on the HP-5MS column.

The compounds were identified by the NIST'08 spectra library (National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.1.2. Antimicrobial Testing of the Essential Oil

The test microorganisms used for evaluation of antimicrobial activity were Gram-positive bacteria (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538), Gram-negative bacteria (*Salmonella abony* NCTC 6017, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 9027), fungal (*Fusarium moniliforme* and *Aspergillus brasiliensis* ATCC 16404) and yeast strains (*Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 2601). The indicator microorganisms were provided by the National Bank for Industrial Microorganisms and Cell Cultures, Bulgaria.

The antimicrobial activity was evaluated by using an agar well diffusion method. Assays were performed in two types of growth media (Tryptic soy agar (TSA) and Sabouraud Dextrose Agar (SDA)). The indicator bacterial strains were grown in TSA (Merk, Germany), while the yeast and fungal strains were grown in SDA (Merk, Germany). A 24 h suspension of the bacterial strains with a density of about 10^7 CFU/mL was added to the media as an inoculant (turbidity: 0.5 McFarland standards). The indicator microorganisms were inoculated into the sterilized media at 50 °C before being poured into sterile Petri plates. A sterile cork borer was used to create 8 mm-diameter wells into which 50 µL of the antimicrobial agent was introduced. The agar plates containing bacterial strains were incubated for 24 h at 37 °C, and those containing yeast and fungal strains were incubated for 72 h at 28 °C. The sensitivity of the test microorganisms to the antimicrobial agent was determined by measuring the distinct zone of growth inhibition around the wells. The diameter of the zones was measured in mm, and values up to 15 mm were considered as poorly sensitive, from 15 to 25 mm—sensitive, and over 25 mm—very sensitive. The assays were performed in parallel with solvent controls [28,29].

2.2. Paper Samples

Paper samples were prepared from cellulose mixtures of bleached kraft woodfree pulp samples from softwood (delivered by SCA, Sweden) and hardwood tree species (delivered by Svilosa AD, Bulgaria) in 80:20 percentage ratio. The used kraft cellulose has been refined separately by laboratory Valley beater method, according to ISO 5264-1:1979, and the value of Schopper Riegler, °SR (ISO 5267-1/AC:2004) of the used cellulose mixture has been 30 °SR.

The analyses have been conducted with one sample of only cellulose, base paper and paper samples treated with lavender oil. we t-end chemical additives have been added to the cellulose mixture and base paper was obtained. The additives were added in the following sequence: alkylketendimer (AKD) sizing agent—1% of o.d.f (Kemira® Fennosize KD 157YC) and cationic retention additive—0.025% of o.d.f. (modified polyacrylamide with molecular weight 11.106 g/mol and charge density +1.05 from Ciba Specialty Chemicals-Ciba® Percol®Co (Basel, Switzerland)).

2.2.1. Microscopic Analysis

Generally, to study the size and structure of the source fibers and to determine paper composition, a specific microscopic determination of cellulose fibers and paper materials is used. According to a standardized methodology described in previous authors' publications [30,31], preparation and analysis of the raw material was carried out. Several small pieces of mechanically pre-milled tested fibers were placed in a porcelain bowl and flooded with 1% NaOH and let stand for 10 min, followed by several times rinsing with distilled water on a fine metal sieve. Fibers were placed and well distributed as three samples on one glass slide. On each of the samples, according to ISO 9184-3:1990, as a colorant is used few drops of Herzberg's reagent (Cl-Zn-I). Fibers should be carefully distributed, free of accumulations and air bubbles, and after drying the samples were covered with a thin glass slide. At 100× magnification with microscope VisiScope® TL254T1 (VWR, Milano, Italy) and objective: 10×/0.25 E-PLAN, Eyepiece: WF10×/20 mm, the obtained colored fiber samples were observed.

2.2.2. The Papermaking Process

Laboratory simulation of the paper manufacturing process was carried out with Rapid-Kothen, Germany laboratory paper-sheet machine, according to ISO 5269-2:2005. Paper samples with basic weight of 50 g/m^2 were obtained and dried at temperature 94 °C for 6 min.

2.2.3. Basic Weight, Thickness, Density, Porosity, Smoothness

Standard method—ISO 536—was used for basic weight determination of all paper samples, followed by thickness determination and density and porosity calculation, as de-scribed in ISO 534 method. Bekk method (ISO 5627/A1:2004) was used for smoothness determination.

2.2.4. Tensile Strength, TEA Index, Elongation and Tear Resistance

On a tensile testing machine Zwick/Roell Z010 (Zwick/Roell GmbH, Ulm, Germany), tensile strength, TEA Index and elongation at break of the obtained paper samples were measured, according to ISO 1924-1/2:2000 at 20 mm/min tested speed. Ten probes of each paper samples with 18 cm length stripes and 1.5 cm in width were used. Tensile strength was determined as tensile index, Nm/g also described in previous authors' publication [32]. The samples were analyzed in the standard atmosphere—23 °C temperature and 50% of relative humidity.

Tear resistance, as described in standard ISO 1974:2012, was performed on an Elmendorf tester and expressed as tear index (with respect to paper basic weight) in mNm^2/g. It measures the force required to tear the paper after a cut was already made. Two parallels were measured for each paper sample.

2.3. Lavender EO Treatment of Obtained Wrapping Paper

Squares of 5 × 5 cm were prepared from the obtained paper samples and weighed. Using a sprayer, the lavender essential oil was applied on both sides of each paper square and let dry at room temperature (25 °C) for 30 min. The dried paper samples were weighed again and the amount of essential oil was calculated. Results indicated that the average amount of the lavender EO is 0.8 mg/cm^2.

2.4. Antimicrobial Testing of Treated Wrapping Paper

A 24 h culture of each indicator bacterial strain was used. Under aseptic conditions, the vegetative material was taken and suspended in 10 mL of sterile saline. The cell concentration of the suspensions was approximately 10^3 CFU/mL. The yeast and fungal strain suspensions were obtained in an analogous manner as for bacterial strains, but with the difference that the cultures were cultivated over the course of 48 h and 120 h for yeast and fungi, respectively. Using sterile tweezers, each square treated with lavender EO was

placed in a sterile Petri dish. On each square, 0.1 mL of the produced cell suspensions were dropped using a sterile pipette and carefully dispersed throughout the surface of the paper before being placed in a thermostat for 2 h at 30–35 °C. In each Petri plate, 20 mL of TSA for bacterial or SDA for yeast and fungal strains were dropped aseptically with a sterile pipette. Control samples included lavender EO and microorganism-free paper and essential oil-free paper with suspension of the current microorganism. In a thermostat, samples were cultured at 30–35 °C for 24–48 h for bacteria and 20–25 °C for 48–72 h and 120 h for yeast and fungi, respectively.

The colonies grown on Petri dishes were counted on a colony counter. By comparing the number of microorganisms grown from each suspension and treated paper with that of the corresponding control samples, the effect of lavender EO-treated paper on the growth of indicator microorganisms was assessed [33,34].

The following equation was used to calculate the efficacy of the antimicrobial effect of the treated paper:

$$\%Efficiency = \frac{N_0 - N_1}{N_0} \times 100 \quad (1)$$

where N_0—number of colony-forming units in the control sample; N_1—number of colony-forming units in the sample paper treated with lavender EO.

3. Results

3.1. Lavender EO Characteristics

The physical properties of the lavender EO are shown in Table 1.

Table 1. Lavender EO characteristics.

Characteristics	Lavender EO
Appearance	Transparent, very mobile liquid
Color	Colorless to pale yellow
Odor	Floral herbaceous scent and balsamic—woody undertone
Relative density, d_{20}^{20}	0.8862 ± 0.05
Refractive index, n_D^{20}	1.4626 ± 0.02
Polarization coefficient, α_D^{20}	-8 ± 0.02
Acid number, (mg KOH/g oil)	0.9 ± 0.04

The gas chromatography (GC) analysis results on the chemical composition of the used lavender EO are presented in Table 2. Thirty-five ingredients were identified in the lavender EO, representing 99.69% of the total amount of substances. Ten of them are in concentrations above 1%, and the remaining twenty-five ingredients are in concentrations below 1%. In an amount above 4% are six compounds: linalyl acetate (36.05%), β-linalool (27.67%), terpinene-4-ol (6.13%), β-cis-ocimene (5.52%), lavandulyl acetate (4.87%), β-caryophyllene (4.68%). According to ISO 3515 /2017, β-trans-ocimene (1.79%) is below the lower limit (2.0%–5.0%) and terpinene-4-ol (6.13%) is above the upper limit (2.0%–5.0%). The main components in lavender oil are linalool and linalyl acetate, and their total amount is 63.72%. In lavender oil, the main compounds are monoterpenes, with esters and alcohols predominating.

3.2. Antimicrobial Activity of Lavender EO

The tested Gram-positive and Gram-negative bacteria were weakly sensitive to the examined lavender EO (Table 3 and Figure 1). The diameter of the zone of growth inhibition in them does not exceed 15 mm.

Table 2. Chemical composition of the lavender EO.

№	Ingredients	RT	Content, %
1.	α-Thujene	9.70	0.15
2.	α-Pinene	9.93	0.31
3.	Camphene	10.47	0.23
4.	β-Pinene	11.60	0.19
5.	3-Octanone	11.74	0.22
6.	Myrcene	11.81	0.53
7.	δ 3-carene	12.42	0.11
8.	Hexyl acetate	12.62	0.07
9.	p-Cymene	13.10	0.57
10.	Limonene	13.23	0.29
11.	Eucalyptol + β-Phellandrene	13.27	0.68
12.	β-cis-Ocimene	13.35	5.52
13.	β-trans-Ocimene	13.67	1.79
14.	cis-Linalyl Oxide	14.54	0.18
15.	trans-Linalyl Oxide	15.05	0.10
16.	β-Linalool	15.55	27.67
17.	1-Octen-3-yl-acetate	15.67	1.28
18.	Camphor	16.90	0.22
19.	Lavandulol	17.40	0.70
20.	Borneol	17.67	0.66
21.	Terpinene-4-ol	17.94	6.13
22.	Cryptone	18.12	0.10
23.	Hexyl isobutyrate	18.18	0.11
24	α-Terpineol	18.35	1.72
25.	Nerol	19.21	0.14
26.	Linalyl acetate	19.96	36.05
27.	Lavandulyl acetate	20.82	4.87
28.	Neryl acetate	22.82	0.51
29.	Geranyl acetate	23.35	0.97
30.	β-Caryophyllene	24.50	4.68
31.	α-Bergamotene	24.78	0.17
32.	(Z)-β-Farnesene	25.25	1.89
33.	α-Caryophyllene	25.40	0.16
34.	Germacrene D	26.03	0.19
35.	Caryophyllene oxide	28.52	0.53
	Total		99.69

Table 3. Antimicrobial activity of lavender essential oil (*Lavandula angustifolia* Mill.).

Sample		Tested Microorganisms	Inhibition Zone (mm)
A	Gram-positive bacteria	*Staphylococcus aureus*	13.9 ± 0.2
B		*Bacillus cereus*	14.5 ± 0.4
C	Gram-negative bacteria	*Escherichia coli*	12.4 ± 0.1
D		*Pseudomonas aeruginosa*	11.7 ± 0.5
E		*Salmonella abony*	11.1 ± 0.1
F	Yeast	*Saccharomyces cerevisiae*	21.0 ± 0.2
G		*Candida albicans*	18.6 ± 0.3
H	Fungal strain	*Aspergillus brasiliensis*	17.0 ± 0.5
I		*Fusarium moniliforme*	15.0 ± 0.5

3.3. Cellulose Fiber Characterization

As wrapping paper for packaging application, the examined paper samples require an excellent strength and optimal surface properties. Hence, bleached virgin kraft cellulose from soft and hardwood tree species was used as a cellulose material. In order to establish

the exact type of wood, a microscopic analysis was carried out through a microscopic analysis illustrated in Figure 2.

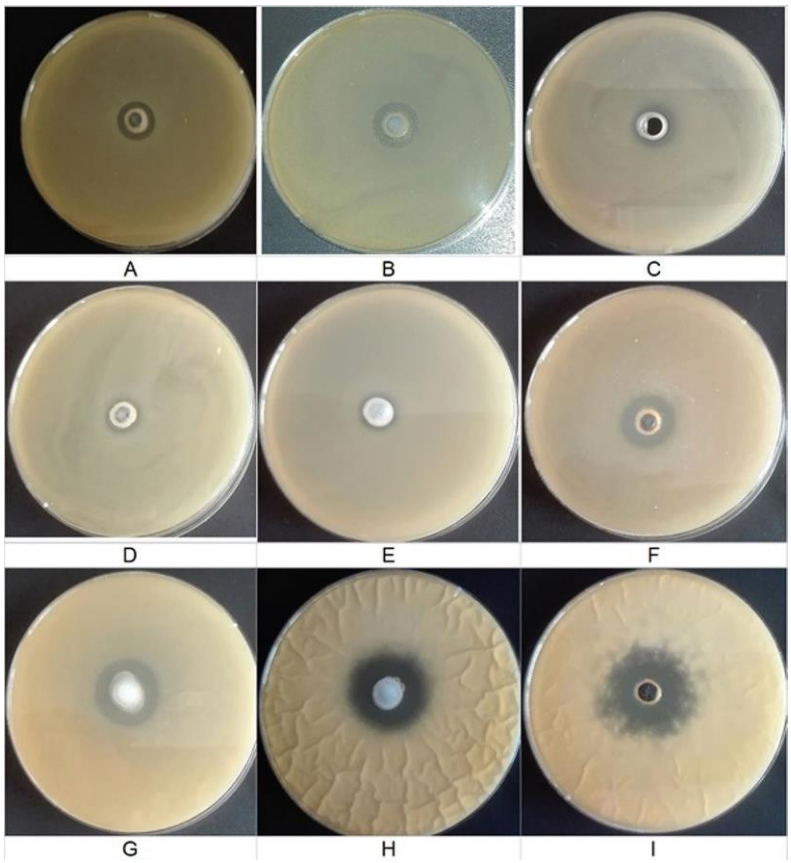

Figure 1. Antimicrobial activity of lavender oil. (**A**)—*Staphylococcus aureus*; (**B**)—*Bacillus cereus*; (**C**)—*Escherichia coli*; (**D**)—*Pseudomonas aeruginosa*; (**E**)—*Salmonella abony*; (**F**)—*Saccharomyces cerevisiae*; (**G**)—*Candida albicans*; (**H**)—*Aspergillus brasiliensis*; (**I**)—*Fusarium moniliforme*.

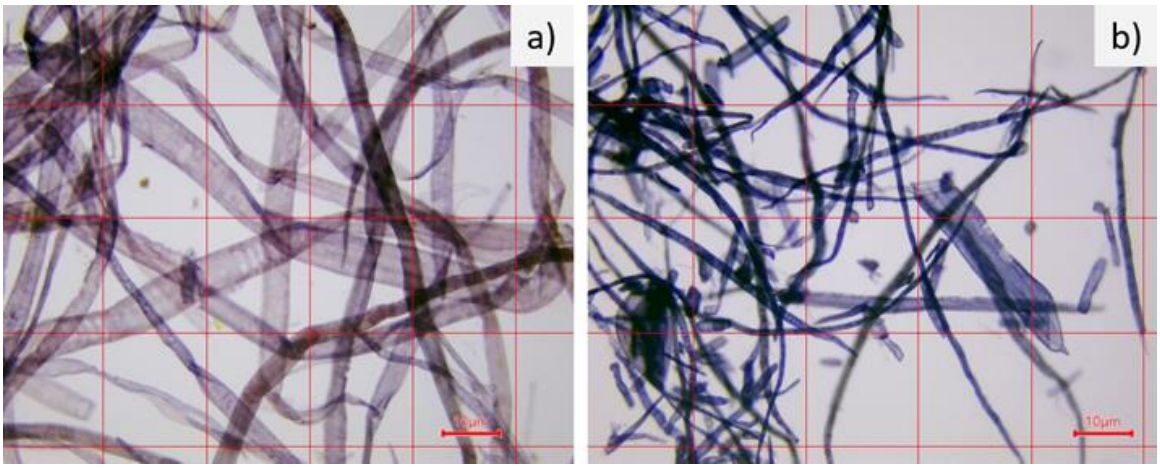

Figure 2. Microscopic photograph of cellulose material: (**a**) sulfate bleached softwood cellulose from pine wood; (**b**) sulfate bleached hardwood cellulose from beech wood.

3.4. Base Paper and Treated Paper Characterization

As an anisotropic material and on the basis of the used experimental methods determining the properties of the paper in different directions, the provenance of meaningful trends is a complex process, which starts with determining the basic weight, thickness,

density, porosity and smoothness of the paper (Table 4). The smoothness of the paper is of essential importance for the surface-treated paper. It is also an indicator of a change in the structure of the paper surface.

Table 4. Basic weight, thickness, density, porosity and smoothness of base and treated paper samples.

Paper Properties	Testing Method		Only Pulp	Base Paper	Lavender-Treated Paper
Basic weight	ISO 536:2012	g/m^2	50.89	50.96	50.88
Thickness	ISO 534:2011	mm	0.8	0.8	0.8
Density	ISO 534:2011	kg/m^3	63.61	63.73	63.42
Porosity	ISO 534:2011	%	95.76	95.75	96.76
Smoothness (Bekk, top side)	ISO 5627/A1:2004	s	10.98	10.96	10.89

The data in Table 5 for the strength properties shows the expected improvement in the four examined indicators: tensile index (Tensile Index, Nm/g), the index of tensile work (TEA Index, mJ/g), the elongation (Elongation, %) and tear index (Tear Index, $mN.m^2/g$) of the base papers compared to cellulose samples as a result of the added wet-end additives—AKD for hydrophobicity of the paper and modified PAA as a retention.

Table 5. Strength properties of base and treated paper samples.

Sample	Composition	Tensile Index, Nm/g	TEA Index, mJ/g	Elongation, %	Tear Index, $Mn{\cdot}m^2/g$
0	Only pulp	63.0	1070	2.4	1.1004
1	Base paper	69.5	1430	2.9	1.0989
2	Lavender-treated paper	65.6	1210	2.6	1.1084

3.5. Antimicrobial Activity of Paper Treated with Lavender EO

In this research, lavender essential oil treatment of wrapping paper is examined for its inhibition activity under nine microorganisms—two Gram-positive bacteria and three Gram-negative bacteria (Figure 3), two yeast and two fungal strains (Figure 4).

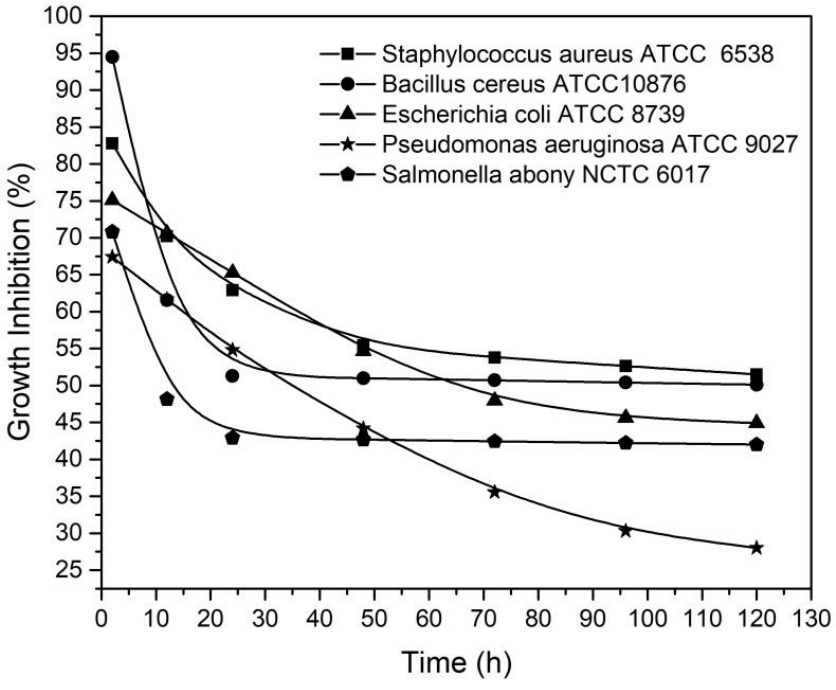

Figure 3. Growth inhibition of the lavender EO-treated paper against Gram-positive and Gram-negative bacteria over time.

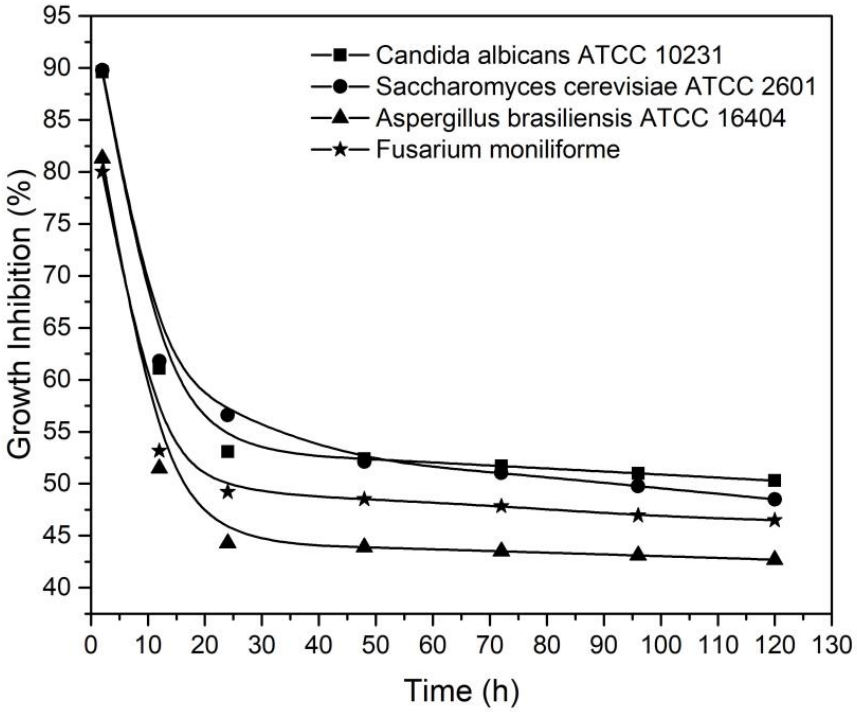

Figure 4. Growth inhibition of the lavender EO treated-paper against the examined yeast and fungal strain over time.

4. Discussion

Lavender EOs with different geographical origins differ in the chemical composition; for example, oil from southern Romania has a higher content of β-linalool (47.55%), camphor (9.67%) and borneol (8.52%) and lower linalyl acetate (3.75%), which is 10 times less than its amount in the Bulgarian lavender EO [12]. Lavender EO from Montenegro [35] has a lower content of linalyl acetate (22.39%) and contains 18.13% 1,8 cineole, which is absent in the Bulgarian essential oil. Polish lavender EO [22], out of a total of 29 compounds, contains more linalool (34.1%) than Bulgarian and Montenegrin and less than Romanian, but the linalyl acetate (33.3%) content is higher compared to Romanian and Montenegrin oil. In South African oil [23], linalyl acetate predominates (36.7%), its amount being close to the Bulgarian one, followed by linalool (31.4%) and terpinene-4-ol (14.9%), whose content is higher compared to Bulgarian lavender oil.

The lavender oil has a greater effect on Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 (13.9 mm) and *Bacillus cereus* ATCC 10 876 (14.5 mm) and lesser effect on Gram-negative *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739 and *Salmonella abony* NCTC 6017.

Lavender oil inhibits the growth of the yeasts *Saccharomyces cerevisiae* ATCC 9763 and *Candida albicans* ATCC 10231 and the fungal strains *Aspergillus brasiliensis* ATCC16404 and *Fusarium moniliforme*. The antifungal activity is stronger against *Aspergillus brasiliensis* ATCC16404 and is slightly weaker against *Fusarium moniliforme*. A different activity of the essential oil was observed against the tested bacterial and fungal strains. This difference is due to the origin and composition of lavender oil, in which oxygenated terpene compounds predominate. In the lavender oil with which the present experiments were carried out, linalyl acetate predominates, which enhances the antifungal effect. From literature [12] it is evident that oils containing less linalyl acetate and more linalool have better antibacterial activity. The predominant terpene compounds in the composition of lavender essential oil determine its antimicrobial activity. With the different origins of the oils, differences in the content of individual components are observed [12,20,22]. Therefore, lavender oil can have different effects on bacterial and fungal strains.

Stoyanova et al. [19,20] found out that Bulgarian lavender oils have an antimicrobial effect on Gram-positive bacteria. Our results show better fungicidal activity of the used lavender oil, explained by the origin as well as the soil-climatic conditions of lavender growth. In other studies [12], lavender oil from southern Romania showed stronger antimicrobial activity against the Gram-negative bacteria *E. coli.* Lavender oil from Montenegro also exhibits antibacterial activity [35]. These differences are due to differences in the composition of lavender oil depending on its origin.

The desired paper properties should correlate with its use in practice and to the certain end use product. As an anisotropic and multifunctional material, paper properties are a result of the variety of its cellulose composition, the wet-end chemical additives used, the production technology and the additional processes used, such as ennobling through coating and callandering.

At the Herzberg's reagent usage, the color of the stained cellulose fibers visualizes the cellulose delignification degree: violet blue color determines the lignin content of up to 9%, while greenish yellow determines that of over 9%. It is also well known that the higher the cellulose delignification degree, the higher and stronger are the formed inter-fiber hydrogen bonds. As it is seen from the microscopic photograph of the used cellulose fibers, the delignification degree is up to 9% (fibers are violet, blue-stained), so it could be summarized that the tensile and tear resistance of the obtained paper samples are going to perform the end users' product requirements.

From the data on the structural–dimensional properties (Table 4) of the base paper and the lavender-treated papers, it is determined that the addition of sizing agent and retention additive does not affect the smoothness of the paper. As expected after the lavender EO treatment, the properties of the samples had mostly the same values with insignificant decrease due to the additional moistening while spraying the lavender EO onto the paper surface.

The lavender EO treatment has a negative effect on the strength properties, but the reduction remains on the level of the strength of the paper samples with a composition of only cellulose. However, the strength of the paper after treatment does not cause further deterioration, and the ability of the cellulose fibers to bond with each other is preserved. This is due to the irreversible destruction of the already formed hydrogen bonds between the cellulose fibers in the paper base, and the subsequent drying after lavender treatment cannot compensate for the loss of the initially formed hydrogen bonds. Therefore, any additional processing of the paper should be carried out before the complete drying of the paper web, and if it cannot be avoided, the decrease in the strength indicators of the paper should be foreseen. In industrial paper production, this reduction could be prevented.

The presented antimicrobial activity results of paper treated with lavender EO show that, 2 h after the lavender treatment, the growth inhibition of the used test microorganisms is between 60–90%. Among the Gram-positive bacteria *S. aureus*, *B. cereus* was 82.8% and 94.5%. Suppression of the growth of Gram-negative bacteria was the following: *E. coli*—75.1%, *P. aeruginosa*—67.4%, *S. ebony*—70.8%. The growth of the yeasts *C. albicans*, *S. cerevisiae* and the molds *A. brasiliensis* and *F. moniliforme* was suppressed by 80%–90%.

After 24 h, the antimicrobial activity of lavender oil-treated paper decreased to 62.9% and 51.3% against Gram-positive bacteria *S. aureus* and *B. cereus*; against Gram-negative bacteria, it was approximately 67.2% for *E. coli*, 54.8% for *P. aeruginosa* and 42.9% for *S. ebony*. The paper's effectiveness against yeasts and molds also decreased, reaching 53.1% against *C. albicans* and 56.6% against *S. cerevisiae*, decreasing to 44.3% against *A. brasiliensis* and up to 49.2% against *F. moniliforme.*

Storing the treated paper for 120 h also results in a decrease in its antimicrobial effectiveness. Against Gram-positive bacteria *S. aureus* and *B. cereus*, it is about 50%. Regarding the Gram-negative bacteria *E. coli*, (44.9%), *P. aeruginosa* (28.0%) and *S. ebony* (42.0%) it is between 20% and 45%. Activity against yeast decreased to about 50%, with *C. albicans* being 50.3% and *S. cerevisiae* being 48.5%. Against the molds *A. Brasiliensis* and *F. monili-*

forme, the effectiveness remains between 40% and 50%. *Aspergillus brasiliensis* and *Fusarium moniliforme* are representatives of different genera of mold fungi, which also determines the differences in the structure and physiology of their cells. This determines the different sensitivity of these microorganisms to lavender essential oil.

5. Conclusions

The lavender EO treatment of wrapping paper has a promising perspective for preserving products from microbial spoilage. Results indicate that wrapping paper treatment with Bulgarian lavender essential oil is successful and the antifungal effect of the obtained treated paper is more pronounced than the antibacterial one due to the linalyl acetate predominance rather than linalool. The antimicrobial efficiency of the obtained treated paper is between 60 and 90% in the first two hours of the treatment, and it gradually decreases to 40%–50% at the end of the five-day period. After the lavender EO treatment, the properties of the paper insignificantly decrease due to the additional moistening, but the ability of the cellulose fibers to bond with each other is preserved and the strength is optimal for further processing. The advantages of the lavender treatment could ensure a broad application in the packaging industry, where the products are subjected to a large variety of biological contaminants.

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