

## ANTIMICROBIAL ACTIVE PACKAGING PAPER BASED ON DILL WEED ESSENTIAL OIL

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Antimicrobial active packaging based on dill weed essential oil (DWEEO) was investigated. Three types of packaging paper (100% bleached pulp 40 g/m<sup>2</sup>, 100% unbleached pulp 40 g/m<sup>2</sup>, and 100% recycled paper weighing 70 g/m<sup>2</sup>) were analysed. The antimicrobial activity was tested against the Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633; the Gram-negative bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NTCC 6017; the yeast *Saccharomyces cerevisiae* ATCC 2601 and *Candida albicans* ATCC 10231; and the fungal strain *Aspergillus brasiliensis* ATCC 16404. The activity of the bleached paper treated with DWEEO against moulds (*Aspergillus brasiliensis*) and yeasts (*Candida albicans*) during the five-day study period decreased from 100 to 47%, against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) decreased from 100 to 69%, and against Gram-negative bacteria (*Salmonella abony* and *Escherichia coli*) decreased from 76 to 12%. Unbleached paper, treated with DWEEO, had stronger antimicrobial potential against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), compared to other test microorganisms. Recycled paper treated with DWEEO showed fungicidal activity.

**Keywords:** antimicrobial activity, active packaging, dill weed essential oil

### INTRODUCTION

Food safety and increasing the shelf life of food products are among the new trends of packaging technologies.<sup>1</sup> However, the permeability of packaging materials does not allow the original composition of the packaging environment to be retained for a long time. The use of high-barrier packaging materials, at an additional expense, was in many cases ineffective, because of the reduced quality of the thermal seals when closing and sealing.<sup>2,3</sup> The most commonly used methods for extending the shelf life of food products are pasteurization, sterilization, drying, freezing *etc.*<sup>4,5</sup> The market supply of packaged food is bound to meet certain requirements concerning the packaging materials and packaging type used.<sup>6,7</sup>

Active packaging is an innovative concept in the food packaging industry, introduced as a result of changes in consumer's requirements and market trends. It performs some functions that conventional packing systems cannot. Antimicrobial packaging is being developed as part of the concept of active packaging. It aims, on the one hand, to inhibit pathogenic microorganisms to ensure the safety of the food and, on the other, to prolong the shelf life of the food and protect it against changes in its organoleptic characteristics.<sup>8</sup> As antimicrobial substances can come into contact with or migrate into the packaged food, the use of plant extracts and their essential oils is preferred in the

development of new food products and nutritional supplements, but also of novel active packaging systems.

The antimicrobial agents that can be used in this type of packaging are divided into three main groups: synthetic, natural (isolated from plants or animals) substances, and probiotics. The antimicrobials occurring naturally have several advantages because they are considered to carry less risk to the consumer.<sup>9-11</sup> Due to their high antimicrobial activity, the interest in the use of essential oils in packaging systems has increased in recent years. Mixtures of essential oils obtained from spices have been incorporated into biodegradable broccoli packages and good antimicrobial effectiveness has been demonstrated against foodborne pathogens *Salmonella typhimurium*, *Escherichia coli* and *Listeria monocitogenes*.<sup>12</sup>

Paper and paperboard are widely used for the packaging of food and beverages. Paper is biodegradable and may include substances that improve its barrier properties.<sup>13-15</sup> Besides, these agents can enhance the safety or sensory qualities of a packaged product while maintaining its quality.<sup>16-20</sup>

Dill weed essential oil (DWEO) is produced in greater quantities than the fruit. The main manufacturers are the USA, France, Hungary, and the countries of Eastern Europe. The DWEO is a pale yellow to yellow, transparent liquid, with a characteristic odor. It is soluble in glyceride and mineral oils, as well as in propylene glycol with opalescence, while it is insoluble in glycerol.<sup>21</sup> The determiners of the odor and taste of the oil are: phellandrene, limonene, dill ether, and carvone. The main constituents of the dill weed essential oil are the following:  $\alpha$ -phellandrene (17.0-66.5%), limonene (5.7-45.0%), carvone (4.3-55.0%), dill ether (2.8-37.5%), and dihydrocarvone (16.0%). The chemical composition of the oil varies depending on the origin of the plant.<sup>22-27</sup>

Previous research demonstrated that DWEO has an antimicrobial effect against various types of microorganisms.<sup>24,27</sup> Therefore, the present study aimed to develop paper-based packaging materials treated with DWEO and investigate their antimicrobial activity.

## EXPERIMENTAL

### Materials and methods

#### Packaging papers

Three types of wrapping paper, which are generally used for food packaging, were used as follows: 100%

recycled paper weighing 70 g/m<sup>2</sup>, papers made from 100% bleached pulp 40 g/m<sup>2</sup> and from 100% unbleached pulp 40 g/m<sup>2</sup>. Microscopic analysis was performed to demonstrate the composition of the fibrous material from which the packaging papers were obtained. From the physico-mechanical properties, the length of tear of the test paper samples was determined. The samples used were analyzed before and after the treatment with DWEO.<sup>28</sup>

#### Essential oil

The DWEO was provided by a manufacturer from Bulgaria. The physical and chemical parameters (appearance, color, odor, relative density, refraction and acid number) of the DWEO were determined.

GC-MS analysis was carried out to find out the chemical composition of the oil. To this end, an Agilent 5975C MSD system, coupled to an Agilent 7890A gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) was used. An Agilent J&W HP-5MS column (0.25  $\mu$ m, 30 m x 0.25 mm) was used with helium as a carrier gas (1.0 mL min<sup>-1</sup>). The operational conditions were the following: oven temperature 35 °C/3 min, 5 °C/min to 250 °C for 3 min, total run time 49 min; injector temperature 260 °C; ionization voltage 70 eV; ion source temperature 230 °C; transfer line temperature 280 °C; solvent delay 4.25 min and mass range 50-550 Da. The MS was operated in the scan mode. One  $\mu$ L of the DWEO was injected into the GC/MS system at a split ratio of 30:1. The GC analysis was carried out using an Agilent 7890A GC system; FID temperature 270 °C. In order to obtain the same elution order with GC/MS, simultaneous triplicate injections were done by using the same column and the same operational conditions.

The identification of compounds was made by comparing their mass spectra with those from mass spectra libraries, as well as by comparing the literature data and estimated Kovat's (retention) indices that were determined using mixtures of homologous series of normal alkanes from C<sub>8</sub> to C<sub>40</sub> in hexane, under the conditions described above. The percentage ratio of volatile components was computed using the normalization method of the GC/FID peak areas.

#### Determination of antibacterial activity of DWEO

The antibacterial activity of (DWEO) was tested against test microorganisms provided by the National Bank for Industrial Microorganisms and Cell Cultures in Sofia, Bulgaria: Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633; Gram-negative bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, and *Salmonella abony* NTCC 6017; yeasts *Saccharomyces cerevisiae* ATCC 2601 and *Candida albicans* ATCC 10231; and fungal strain *Aspergillus brasiliensis* ATCC 16404.

The antimicrobial activity was determined by the agar well diffusion method, using a well size of 8 mm.

The growth media were tryptic soy agar (Merck) for the tested bacterial strains and Sabouraud dextrose agar (Merck) for the yeasts and fungi. The media were inoculated with a 24-hours suspension of the bacterial species, with a density of approximately  $10^7$  CFU/mL (turbidity: 0.5 McFarland standards). Media melted and cooled to 50 °C were inoculated with the tested microorganisms and then equally dispensed into Petri dishes. Next, a hole with a diameter of 8 mm was punched aseptically with a sterile cork borer, and a volume (50  $\mu$ L) of the antimicrobial agent was introduced into the well. After that, the agar plates were incubated at 37 °C or 28 °C for 24 or 72 hours according to the microbial species. After cultivation, the distinct zone of growth inhibition around the wells was measured using a digital caliper. The diameter of the zones, including the diameter of the well, was recorded in mm, for instance, up to 15 mm the microbial culture was poorly sensitive, from 15 to 25 mm it was considered sensitive, and over 25 mm it was considered very sensitive. The tests were performed in parallel with solvent controls.<sup>29</sup>

#### Determination of the antimicrobial properties of paper treated with DWEO

5/5 cm squares were prepared from each paper. DWEO (approximately 0.05 g/square) was applied with a pump dispenser on both sides of each paper square, which was then dried at room temperature (25 °C) for 10 min. The amount of essential oil used was estimated by the difference in weight of each paper square before and after the application of the DWEO. The antimicrobial activity of treated papers was examined after 2 hours, 24 hours and 5 days.

A 24-hours culture was prepared from each bacterial test microorganism. With a wire loop, vegetative material was taken and suspended in 10 mL of saline. The suspensions prepared had a cell concentration of about  $10^3$  CFU/mL. Yeast and mould suspensions were prepared in the same manner, but the cultures used were at the age of 48 hours for the yeast and 120 hours for the mould.

In aseptic conditions with sterile tweezers, each square treated with DWEO was placed in a sterile Petri dish. On each square, 0.1 mL of the prepared cell suspensions was dropped with a sterile pipette and carefully spread over the surface of the paper. Then, the Petri dishes were placed in a thermostat at 30-35 °C for 2 hours. Aseptically, with a sterile pipette, 20 mL of tryptic soy agar, for the bacteria, or Sabouraud-dextrose agar, for the yeast and moulds, was dropped in every Petri dish.

The following control samples were also prepared: DWEO and microorganism free paper and DWEO free paper with a suspension of the respective microorganism.

Samples were cultured in a thermostat at 30-35 °C for 24-48 hours for bacteria and at 20-25 °C for 48-72

hours for the yeast and for 120 hours for the mould. The colonies grown in the Petri dishes were counted.

The influence of the paper treated with DWEO on the growth of the test microorganisms was evaluated by comparing the number of microorganisms from each suspension and the treated paper with the respective control samples.

The efficiency of the antimicrobial effect of the paper treated with DWEO was calculated by the formula:

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

where  $N_0$  – number of colony forming units in the control sample;  $N_t$  – number of colony forming units in the sample paper treated with DWEO.

## RESULTS AND DISCUSSION

The characteristics of DWEO are shown in Table 1. The DWEO is a colored liquid with a typical odor, which is characteristic of the aromatic compound carvone. The other properties of the DWEO are in agreement with the results reported in the literature.<sup>21,23</sup>

The chemical composition of the DWEO is presented in Table 2. There were identified 26 constituents in the DWEO, representing 98.94% of the total content. Eight of them were in concentrations above 1% and the rest 18 constituents were in concentrations under 1%. The main constituents in the solution (above 3%) were: carvone (32.26%),  $\alpha$ -phellandrene (20.11%), limonene (18.78%), dill ether (9.06%), and *p*-cymene (4.40%). The distribution of the major groups of aroma substances in the DWEO is shown in Table 2. Monoterpene hydrocarbons and oxygenated monoterpenes were the dominant groups in the DWEO. The oil sample analyzed in this study exhibited a chemical profile similar to that reported in the literature, with identical qualitative composition and only some minor quantitative differences.<sup>22-27</sup>

A number of aromatic compounds have been registered by the European Commission for use as flavorings in foodstuffs. These compounds are considered to present no risk to the consumers' health and include, amongst others, carvone, *p*-cymene and limonene.<sup>31</sup> The results regarding the properties and the chemical composition of the DWEO reveal its potential use as flavoring in packaging papers for food products.

Microscopic images of the types of paper used are shown in Figure 1. The microscopic method was used to identify the fiber composition using the Hezberg reagent.<sup>28,30</sup> The sulfate cellulose is colored in purple with a brownish tinge. Flat broad

fibers indicate the presence of deciduous wood pulp, while thin and long fibers indicate the presence of sulfate cellulose from coniferous wood. Yellow coloration indicates the presence of lignin and wood pulp.

Table 3 shows that the papers treated with DWEO changed their properties. There was a

decrease in the physico-mechanical parameters of the paper samples (breaking length by about 200 m). This indicated that a loss of hydrogen bonds occurred when the DWEO penetrated the paper. However, these changes were not significant and the papers could be used as intended.

Table 1  
Indicators of DWEO

Indicators	DWEO
Appearance	liquid
Color	light yellow
Odor	characteristic, spicy-dill
Relative density ( $d_{20}^{20}$ )	$0.8979 \pm 0.00$
Refractive index ( $n_D^{20}$ )	$1.4832 \pm 0.01$
Acid number, (mg KOH/g oil)	$1.24 \pm 0.01$

Table 2  
Chemical composition of the DWEO

№	Compounds	RI	Content, %
1.	$\alpha$ -Thujene	924	$0.30 \pm 0.00$
2.	$\alpha$ -Pinene	932	$2.22 \pm 0.02$
3.	Camphene	946	$0.17 \pm 0.00$
4.	Sabinene	969	$0.39 \pm 0.00$
5.	$\beta$ -Pinene	975	$0.73 \pm 0.00$
6.	Myrcene	988	$0.70 \pm 0.00$
7.	$\alpha$ -Phellandrene	1001	$20.11 \pm 0.19$
8.	p-Cymene	1020	$4.40 \pm 0.04$
9.	Limonene	1025	$18.78 \pm 0.17$
10.	Terpinolene	1084	$0.15 \pm 0.00$
11.	p-Cymenene	1091	$0.27 \pm 0.00$
12.	$\beta$ -Linalool	1096	$0.18 \pm 0.00$
13.	p-Cymen-8-ol	1178	$0.34 \pm 0.00$
14.	Dill ether	1184	$9.06 \pm 0.00$
15.	Methyl chavicol	1193	$1.91 \pm 0.01$
16.	trans-Dihydrocarvone	1200	$2.90 \pm 0.02$
17.	p-Cymen-9-ol	1205	$0.41 \pm 0.00$
18.	cis-Carveol	1226	$0.51 \pm 0.00$
19.	Carvone	1237	$32.26 \pm 0.30$
20.	cis-Carvone oxide	1259	$0.21 \pm 0.00$
21.	trans-Carvone oxide	1270	$0.17 \pm 0.00$
22.	Limonen-10-ol	1287	$0.78 \pm 0.00$
23.	Carvacrol	1298	$0.69 \pm 0.00$
24.	cis-2,3-Pinenediol	1307	$0.86 \pm 0.00$
25.	iso-Dihydro carveol acetate	1326	$0.31 \pm 0.00$
26.	Decyl acetate	1407	$0.13 \pm 0.00$
	Aliphatic hydrocarbons, %		0.13
	Monoterpene hydrocarbons, %		44.02
	Oxygenated monoterpenes, %		38.59
	Phenyl propanoids, %		4.72
	Oxygenated phenyl propanoids, %		3.38
	Others, %		9.16

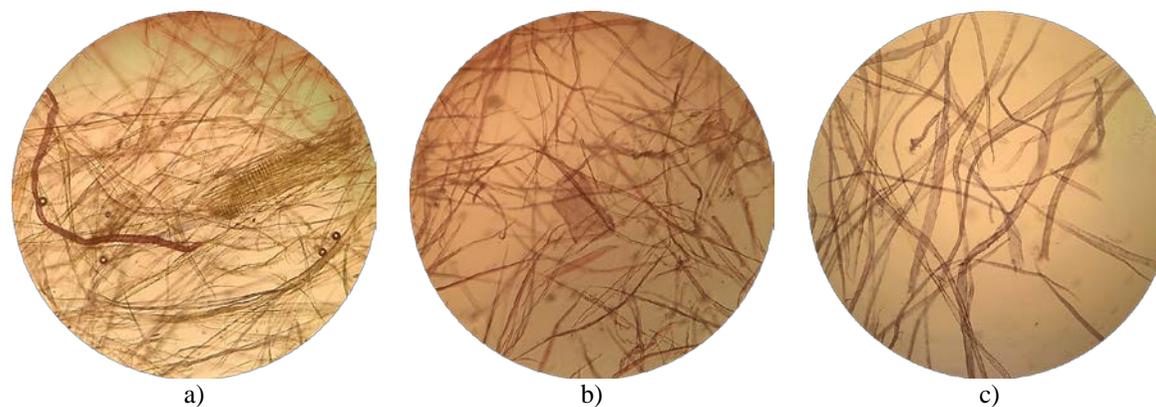


Figure 1: Packaging paper made from: a) recycled, b) bleached cellulose, and c) unbleached cellulose

Table 3  
Physico-mechanical parameters of the paper samples

Parameter	Bleached cellulose				Unbleached cellulose				Recycled paper			
	Length of tear in longitudinal direction	Relative longitudinal extension	Length of transverse rupture	Relative extension in transverse direction	Length of tear in longitudinal direction	Relative longitudinal extension	Length of transverse rupture	Relative extension in transverse direction	Length of tear in longitudinal direction	Relative longitudinal extension	Length of transverse rupture	Relative extension in transverse direction
Paper sample	m	%	m	%	m	%	m	%	m	%	M	%
Untreated	2500	1.4	2200	1.6	5800	1.0	1800	1.5	2800	0.8	1900	2.0
Treated with DWEO	2200	1.2	2000	1.4	5600	0.8	1600	1.2	2600	0.6	1600	1.8

**Antimicrobial activity of DWEO**

The DWEO exhibited fungicidal activity against the tested mould and yeasts, with inhibition zone diameters between 16.3 and 25.9 mm (Fig. 2). The effect of the DWEO was less pronounced against Gram-negative bacteria *S. abony* (16.9 mm), *E. coli* (15.8 mm) and *P. aeruginosa* (14.1 mm), as well as against Gram-positive bacteria *S. aureus* (15.1 mm) and *B. subtilis* (14.4 mm). Our results are in agreement with the findings reported in the literature,<sup>24,27</sup> according to which DWEO inhibited Gram-positive and Gram-negative bacteria and possessed an antifungal effect. The established antimicrobial activity of the tested DWEO is explained by the content of oxygen derivatives (carvone and dill ether), which have higher antimicrobial action than hydrocarbons ( $\alpha$ -phellandrene, limonene, and *p*-cymene).<sup>24,32</sup> An important characteristic of DWEO and its major components is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, dissipating the pH gradient and membrane potential of cells.<sup>32</sup>

**Antimicrobial properties of paper treated with DWEO**

The results regarding the antimicrobial activity of paper treated with DWEO are presented in Figures 3, 4 and 5.

In the experiments carried out with bleached cellulose packaging paper (Fig. 3), growth inhibition of all test microorganisms was detected two hours after the application of DWEO. The growth of Gram-positive bacteria was suppressed – for *S. aureus* (100% efficiency) and *B. subtilis* – 83%. After 24 hours, the antimicrobial activity of the paper against these microorganisms was reduced to 94% and 76%, respectively. After 5 days, this effect decreased to 78% for *S. aureus* and 69% for *B. subtilis*.

The packaging paper had a lower inhibitory effect on Gram-negative bacteria two hours after the application of the DWEO. The number of *E. coli* cells decreased by 46% and that of *S. abony* by 76%. After 24 hours, the antimicrobial activity against *E. coli* and *S. abony* was 44% and 71%, respectively.

The 5 day storage period reduced the effectiveness to 12% and 68%, respectively.

Full inhibition (100%) of *C. albicans* yeast growth and of 90% for *A. brasiliensis* mould was observed 2 hours after the DWEO application. After 24 hours, the effectiveness of the antifungal potential decreased to 90% and 76%, respectively. After 5 days, this efficiency was 65% for *C. albicans* and 47% for *A. brasiliensis*.

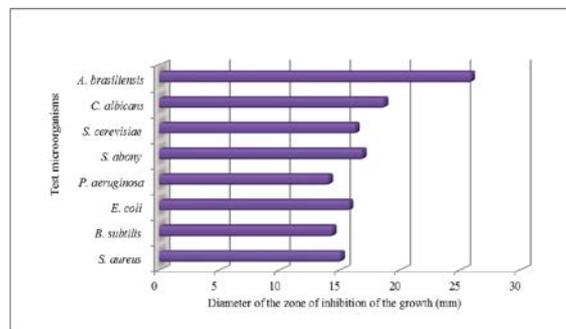


Figure 2: Antimicrobial activity of DWEO

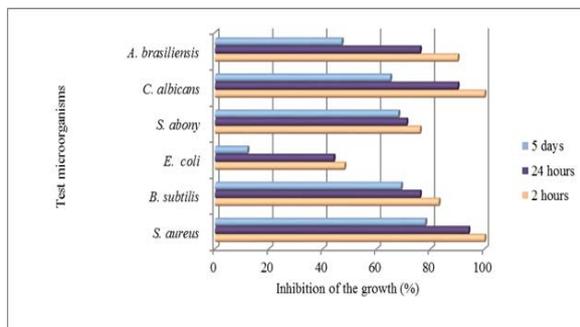


Figure 3: Antimicrobial activity of bleached cellulose packaging paper treated with DWEO

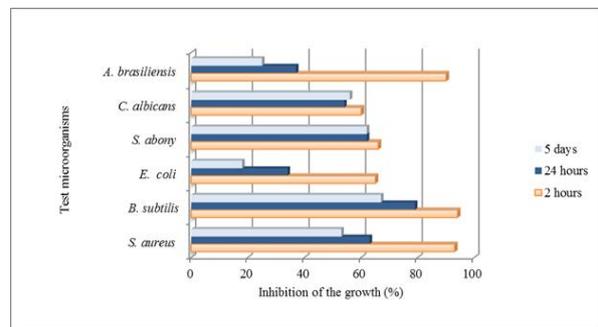


Figure 4: Antimicrobial activity of unbleached cellulose packaging paper treated with DWEO

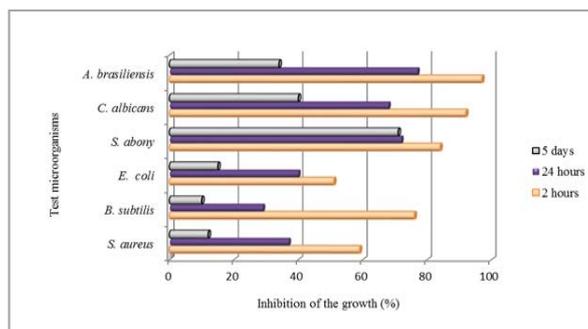


Figure 5: Antimicrobial activity of recycled paper treated with DWEO

The experiments conducted with unbleached cellulose packing paper showed growth inhibition of Gram-positive test microorganisms *S. aureus* (93%) and *B. subtilis* (94%) 2 hours after DWEO was applied (Fig. 4). The 24 hours storage reduced the antimicrobial activity of the paper to 63 and 79%, respectively. After five day storage, the effect decreased to 53% for *S. aureus* and 67% for *B. subtilis*.

Gram-negative bacteria were less affected by the oil-treated unbleached cellulose than Gram-positive ones. After 2 hours, the growth of *E. coli* was reduced to 65% and that of *S. abony* to 66%. After 24 hours and 5 days of paper storage, the antimicrobial activity against *S. abony* did not change significantly (62%), while gradually decreasing to 34% (24 hours) and 18% (5 days) against *E. coli*.

High inhibition (60%) of *C. albicans* cells was detected after 2 hours when DWEO was applied. Storing the paper for 24 hours and 5 days did not significantly reduce its antimicrobial activity (54% and 56%, respectively).

Packaging paper from unbleached pulp inhibited by 90% the growth of *A. brasiliensis* 2 hours after the addition of DWEO. After 24 hours and 5 days, the impact efficiency was of 37 and 25%, respectively.

Two hours after the addition of DWEO, the recycled paper inhibited the growth of Gram-positive test microorganisms *S. aureus* (59%) and *B. subtilis* (76%) (Fig. 5). After 24 hours of storage, the antibacterial action decreased to 37% and 29%, respectively. After five days, the effectiveness against *S. aureus* and *B. subtilis* dropped to 12% and 10%, respectively.

Recycled paper inhibited 51% of *E. coli* growth and 84% of *S. abony* 2 hours after the treatment with DWEO. After 24 hours and 5 days of storage, the antimicrobial action against *S. abony* decreased to 72% and remained at this level. The effectiveness against *E. coli* gradually decreased to

40% after 24 hours and to 15% on day 5 of storage.

The treatment of recycled paper with DWEO resulted in 92% inhibition of *C. albicans* growth and 97% inhibition of *A. brasiliensis*. After 24 hours, the antimicrobial activity decreased to 68% and 77%, respectively. After 5 days, the activity reached 40% for *C. albicans* and 34% for *A. brasiliensis*.

These results can be probably explained by some synergistic/antagonistic component interactions within the DWEO or the problematic solubility in the papers, which have different microfiber composition. The interactions between the DWEO and its components with other paper ingredients or additives need to be investigated in the future.

## CONCLUSION

The antimicrobial activity of three samples of packaging papers (bleached, unbleached and recycled) was analyzed after their treatment with dill weed essential oil (DWEO).

The effectiveness of bleached paper treated with dill weed oil against moulds and yeasts decreased from 100 to 47%, against Gram-positive test microorganisms – from 100 to 69%, and against Gram-negative ones – from 76 to 12%, during the five days of study. Unbleached paper, coated with DWEO, had a better effect on the reduction of the Gram-positive bacteria than on other test microorganisms. Recycled paper treated with DWEO exhibited fungicidal activity.

The results obtained allow the usage of DWEO in food packaging to improve the quality and extend the shelf life of food.

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