Effect of clary sage (*Salvia sclarea* L.) essential oil on paper packaging materials

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Keywords:

Packaging
Paper
Clary sage
Salvia *sclarea*
Essential oil
Antimicrobial

Article history:

Received 14.07.2019
Received in revised form 26.10.2019
Accepted 30.06.2020

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DOI: 10.24263/2304-974X-2020-9-2-3

Abstract

Introduction. The aim of research – to determine the effect of clary sage (*Salvia sclarea* L.) essential oil on paper packaging materials.

Materials and methods. Three packaging materials have been studied based on paper coated with clary sage essential oil. The chemical composition of the clary sage essential oil is determined chromatographically. Antimicrobial effect of essential oil was determined against Gram-positive, Gram-negative bacteria, yeasts, and fungi using the agar diffusion method.

Results and discussion. The chemical composition of the clary sage essential oil showed a predominant amount of oxygenated monoterpenes (83.43%), followed by monoterpene hydrocarbons (7.86%), and sesquiterpene hydrocarbons (5.16%). The major components of the clary sage essential oil were linalyl acetate, β-linalool, α-terpineol, limonene, and geranyl acetate that determined the antimicrobial action of the oil.

The essential oil exhibited a fungicidal action against the tested molds and yeasts. Its high antimicrobial properties could be probably due to the high content of linalyl acetate (40.31%) and *β*-linalool (22.72%).

Our results showed high fungicidal efficacy for the three types of packaging materials. The suppressive action against *C. albicans* during the investigated shelf life period was about 100%. It was found a high efficiency of the recycled paper against *A. brasiliensis* (99.2% - 81.9%). It was determined that the bactericidal effect of the tested packaging materials was lower than the Gram-negative bacterium *S. abony*.

Conclusions. Clary sage essential oil could be used as an antimicrobial agent in the food industry due to its antimicrobial properties, in order to improve the quality of the products and extend their shelf life.

Introduction

Clary sage essential oil is obtained by steam distillation of flowering inflorescences of the plant. The main constituents in the essential oil are linalyl acetate (45– 65%) and *β*-linalool (15–25%). It has a musky, bittersweet smell [1]. It has an antibacterial and antiseptic action [2,3]. It is mainly used in the perfumery, cosmetics, aromatherapy, and phytotherapy. The leaves and inflorescences are used also for flavoring of some foods - meat, dairy, vegetables, *etc*. [1].

The usage of plant extracts and essential oils is preferred in the development of novel active packings due to the ability of the antimicrobial substance to contact the product or to penetrate food [4,5].The interest in the usage of essential oils in packing systems has increased in recent years due to their high antimicrobial activity. An antimicrobial active packaging developed based on packing paper and essential oils has been used successfully in the storage of strawberries [6].

The aim of the present study is to develop a wrapping paper with the clary sage essential oil and to study its antimicrobial efficacy.

Materials and methods

Materials

Packaging papers. Three types of wrapping papers were used - 100% recycled paper weighing 70 g/m^2, 100% bleached pulp 40 g/m^2 and 100% unbleached pulp 40 g/m^2.

Essential oil. The clary sage essential oil was provided by a manufacturer in Bulgaria.

Methods

Paper analysis. 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 have been analyzed before and after treatment with clary sage essential oil [7].

Oil analysis. The physical and chemical parameters (appearance, color, odor, relative density, refraction, and acid number) of the clary sage essential oil were determined [8].

The GC-MS analysis was carried out with an Agilent 5975C MSD system coupled to an Agilent 7890A gas chromatograph (Agilent Technologies Inc., Santa Clara, CA). Agilent J&W HP-5MS column (0.25 μm, 30 m x 0.25 mm) was used with helium as a carrier gas (1.0 mL min^{-1}). The operational conditions were: 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 scan mode. One μL of the sample 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 and by comparing the literature and estimated Kovat's (retention) indices that were determined using mixtures of homologous series of normal alkanes from C_8 to C_{40} in hexane, under the conditions described above [9]. The percentage ratio of volatile components was computed using the normalization method of the GC/FID peak areas.

Determination of antimicrobial activity. The antimicrobial activity of clary sage essential oil 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, *Bacillus subtilis* ATCC 6633; Gram-negative bacteria: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella abony* NTCC 6017; yeast: *Saccharomyces cerevisiae* ATCC 2601, *Candida albicans* ATCC 10231; and fungal strain: *Aspergillus brasiliensis* ATCC 16404.

The antimicrobial activity was determined by the agar well diffusion method with 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 yeast and fungi. The media were inoculated with a 24-h 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 Petry dishes. Next, a hole with a diameter of 8 mm was punched aseptically with a sterile cork borer, and a volume (50 μL) of the antimicrobial agent was placed into the well. After that, the agar plates were incubated at 37 °C or 28 °C for 24 or 72 h 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 as very sensitive. The tests were performed in parallel with solvent controls [10].

Determination of the antimicrobial properties of paper coated with clary sage essential oil. The clary sage essential oil was applied with a pump dispenser on both sides of each paper square (5/5 cm squares) and dried. The antimicrobial activity of coated papers was examined after 2 h, 24 h, and 5 days. A 24 h-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 were with a cell concentration of about 10^3 CFU/mL. Yeast and mold suspensions were prepared in the same manner, but the cultures used were at the age of 48 h for yeast and 120 h for mold. In aseptic conditions with sterile tweezers, each square coated with essential oil was placed in a sterile petri dish. With sterile pipette on each square was dropped 0.1 mL of the prepared cell suspensions and carefully spread over the surface of the paper and then they were placed in a thermostat at 30–35 °C for 2 h. Aseptically with a sterile pipette, in every petri dish were dropped 20 mL of Tryptic soy agar for bacteria or Sabouraud-dextrose agar for yeast and molds. The following control samples were also prepared: clary sage essential oil and microorganism free paper and essential oil free paper with suspension from the current microorganism were obtained. Samples were cultivated in a thermostat at 30–35° C for 24–48 h for bacterial species and at 20–25 °C for 48–72 h for yeast and 120 h for mold. The colonies grown in the petri dishes were encountered [10].

Results and discussion

The clary sage essential oil is a light yellow liquid with musky and bitter-sweet odor.

Indicators as appearance, color, odor, relative density refractive index, and an acid number of lary sage essential oil were presented in Table 1. The results are in agreement with the data found in the literature [1].

Table 1

Indicators of clary sage essential oil

Indicators	Clary sage essential oil
Appearance	Liquid
Color	Light yellow
Odor	Musky, bitter-sweet
Relative density (d_{20}^{20})	0.9034 ± 0.00
Refractive index (n_D^{20})	1.4602 ± 0.05
Acid number (mg KOH/g clary sage essential oil)	1.52 ± 0.02

Chemical composition of clary sage essential oil

The chemical composition of the clary sage essential oil was shown in Table 2.

In the clary sage essential oil were identified 30 constituents representing 98.97% of the total content of the oil as 5 of them were in concentrations above 1% and the rest 25 constituents were in concentrations under 1%. The main constituents in the clary sage essential oil (above 3%) were: linalyl acetate (40.31%), β-linalool (22.72%), α-terpineol (7.74%), limonene (5.40%), and geranyl acetate (4.63%). These constituents determined the antimicrobial properties of the clary sage essential oil [2,3].

The differences between the essential oil yield in this study and that from other studies, reported in the literature [1,11,12] are probably due to the climatic conditions in the respective locality where the plants were grown, and also to the plant parts processed and extracted.

The distribution of major groups of aroma substances in the clary sage essential oil was also shown in Table 2. Oxygenated monoterpenes and monoterpene hydrocarbons dominated in the clary sage essential oil.

The physico-mechanical parameters (length of the tear in the longitudinal direction and relative longitudinal extension) of three different types of papers were presented (Table 3).

The results showed that the paper coatings with clary sage essential oil affected their properties as the length of the tear in the longitudinal direction and relative longitudinal extension. A decrease in the physico-mechanical parameters (breaking length by about 200 m) was indicated. The results showed that the loosening of the hydrogen bonds occurred when the clary sage essential oil has penetrated. These changes were not significant and the papers could be used for their intended purpose.

Table 2

Chemical composition of the clary sage essential oil

Name	RT[1]	RI[2]	Content, % of TIC[3]
α-Pinene	9.69	932	0.22
β-Pinene	11.08	975	0.31
β-Myrcene	11.51	988	1.49
p-Cymene	12.64	1020	0.21
Limonene	12.81	1025	5.40
cis-Linalyl oxide	14.11	1067	0.37
Terpinolene	14.56	1084	0.36
β-Linalool	15.08	1090	22.72
α-Terpineol	17.94	1188	7.74
Linalyl formate	18.40	1215	0.38
β-Citronellol	18.81	1224	0.94
Nerol	19.53	1230	0.41
Linalyl acetate	19.75	1254	40.31
α-Citral	20.04	1268	0.43
Neryl formate	20.25	1280	0.11
Geranyl formate	20.82	1297	0.24
Linalyl propanoate	21.76	1334	0.65
α-Cubebene	22.05	1345	0.88
Citronellyl acetate	22.14	1350	0.85
Neryl acetate	22.46	1361	2.78
Geranyl acetate	23.01	1380	4.63
β-Cubebene	23.19	1386	0.37
β-Bourbonene	23.27	1389	0.23
β-Caryophyllene	24.12	1420	1.54
β-Selinene	25.63	1487	0.28
α-Selinene	25.80	1496	1.81
Spathulenol	28.09	1577	1.92
Caryophyllene oxide	28.58	1582	0.12
(2-Z,6-E)-Farnesyl acetate	34.66	1821	0.20
n-Docosane	40.53	2200	1.07
Total, %			98.97
Aliphatic hydrocarbons, %			1.08
Monoterpene hydrocarbons, %			7.86
Oxygenated monoterpenes, %			83.43
Sesquiterpene hydrocarbons, %			5.16
Oxygenated sesquiterpenes, %			2.26
Phenyl propanoids, %			0.21

1 - retention time, min;
2 - retention (Kovat's) index;
3 - identified at > 0.05% of TIC

Table 3

Length of packing paper cuts

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

Antimicrobial activity of clary sage essential oil

The clary sage essential oil exhibited a fungicidal action against the tested strains of mold and yeast, with an inhibition zone diameter between 16.5 and 23.1 mm (Fig. 1). It was determined a bactericidal effect of clary sage essential oil on Gram-positive bacteria *S. aureus* (18.6 mm) and *B. subtilis* (17.9 mm) as the antimicrobial activity was determined to be lower against Gram-negative bacteria *S. abony* (16.7 mm)), *E. coli* (15.8 mm) and *P. aeruginosa* (14.6 mm). The antimicrobial properties of clary sage essential oil have been studied by other researchers [2,3]. We consider that there is a correlation between the antimicrobial activity of clary sage essential oil and its chemical composition [3, 13]. Its high antimicrobial properties against the investigated test-microorganisms could be probably due to the high content of linalyl acetate (40.31%) and *β*-linalool (22.72%).

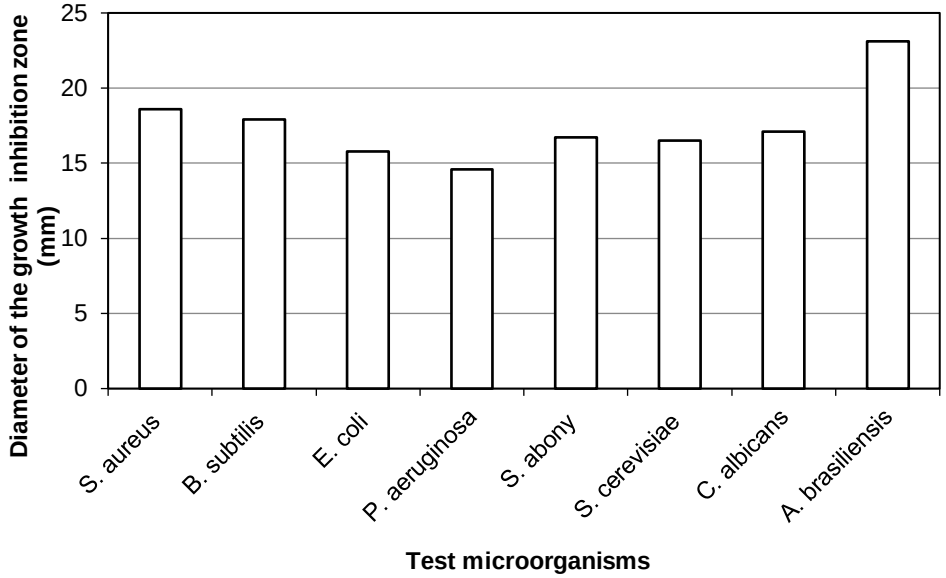

Figure 1. Antimicrobial activity of clary sage essential oil

Antimicrobial properties of paper coated with clary sage essential oil

The antimicrobial activity of different papers coated with clary sage essential oil was presented (Figure 2, 3 and 4).

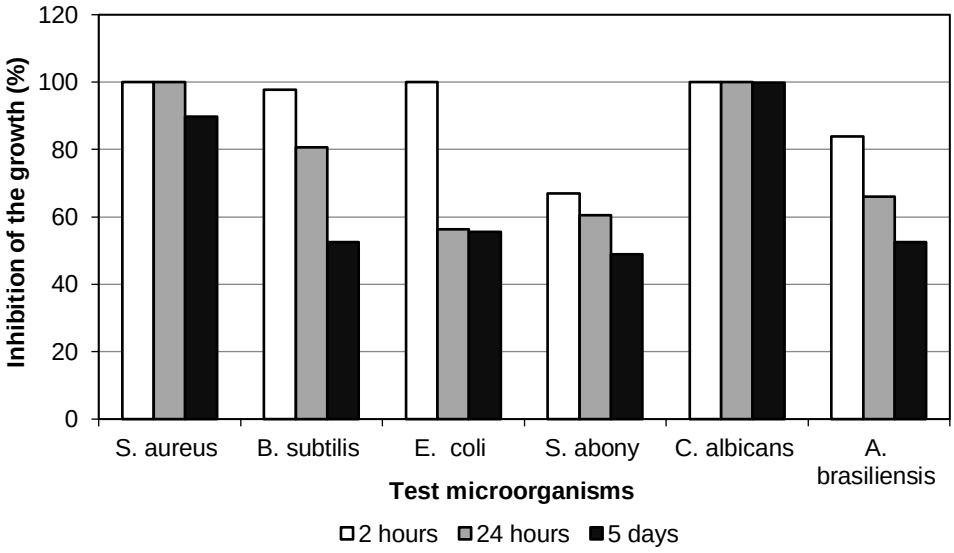

Figure 2. The antimicrobial effectiveness of bleached cellulose wrapping paper coated with clary sage essential oil

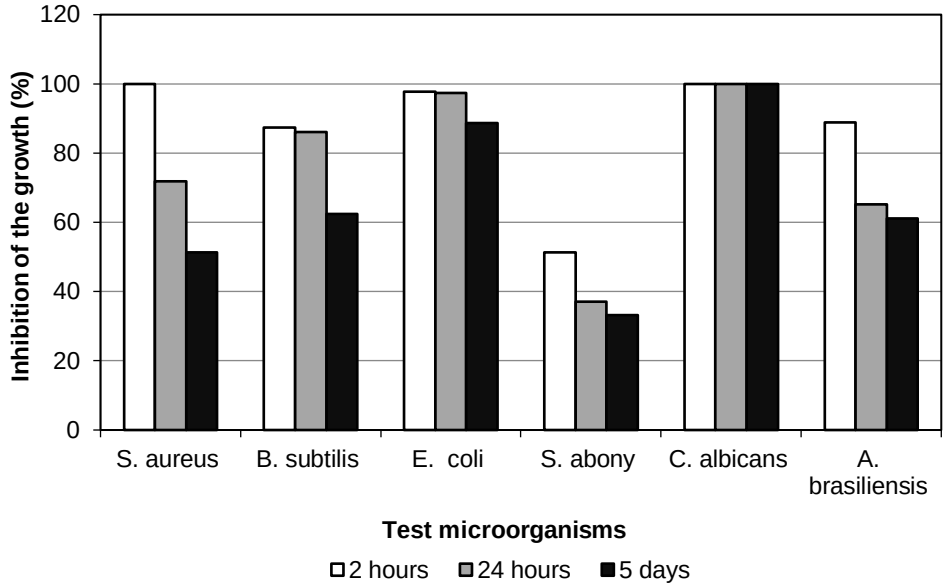

Figure 3. The antimicrobial effectiveness of unbleached cellulose wrapping paper coated with clary sage essential oil

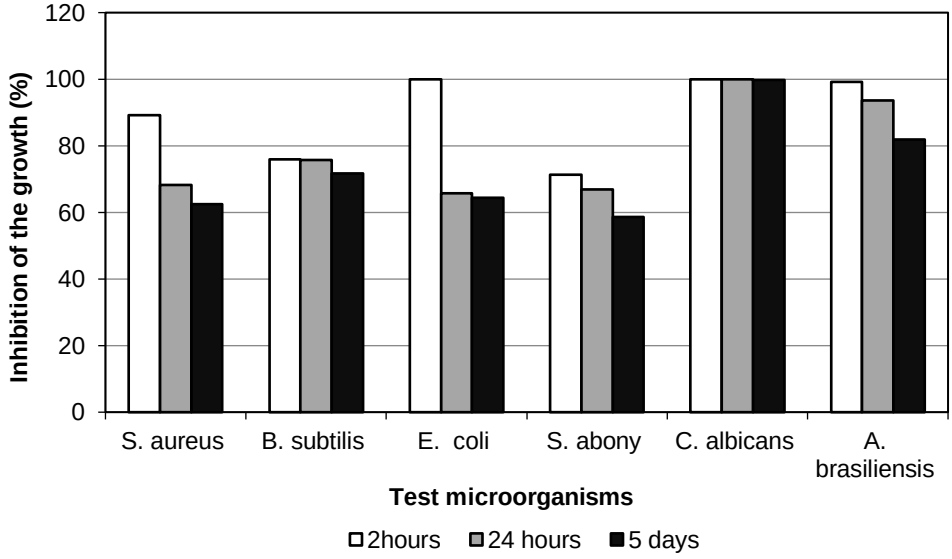

Figure 4. The antimicrobial effectiveness of recycled paper coated with clary sage essential oil

The results obtained two hours after application of the clary sage essential oil to bleached cellulose wrapping paper (Figure 2) showed inhibitory action of *S. aureus* growth (100% efficiency) and *B. subtilis* (97.8%). Keeping the paper for 24 h did not affect its antimicrobial action against *S. aureus*, but it was decreased against *B. subtilis* (80.6%). After 5 days of storage, antimicrobial effect decreased to 89.8% for *S. aureus* and 52.5% for *B. subtilis*.

Packaging paper had a full inhibitory effect on *E. coli* (100%) two hours after clary sage essential oil application and weaker activity for *S. abony*, where viable cells decreased to 66.9%. After 24 h-storage, the antimicrobial action against *E. coli* and *S. abony* was found to be 56.3% and 60.5%, respectively. The five-day storage period reduced the antimicrobial effectiveness to 48.9% for *S. abony*.

Full inhibition of *C. albicans* yeast growth and 83.9% in the *A. brasiliensis* mold growth was observed 2 h after the clary sage essential oil application. After 24 h, the antimicrobial activity remained 100% in *C. albicans* and decreased to 66% in *A. brasiliensis*. After 5 days, this efficiency was 99.8% for *C. albicans* and 52.6% for *A. brasiliensis*.

Our results for the unbleached cellulose wrapping paper (Figure 3) showed an inhibition of the growth of Gram-positive test organisms *S. aureus* (100%) and *B. subtilis* (87.3%) two hours after the addition of clary sage essential oil. Keeping the paper for 24 h reduced the antibacterial action to 71.8 and 86.1%, respectively. After a five-day storage period, it was determined that the antimicrobial effectiveness was 51.3% against *S. aureus* and 62.4% against *B. subtilis*.

Among the Gram-negative bacteria, *S. abony* was less affected than *E. coli* by unbleached cellulose paper. After 2 h of storage, the growth of *E. coli* was reduced to 97.8%, while the growth of *S. abony* was found to be 51.3%. After 24 h of paper storage, the effectiveness of the antimicrobial action against *E. coli* did not change significantly and was 97.3%, while it was determined to be decreased to 37.1% for *S. abony*. Our results showed that the antimicrobial action was decreased to 88.7% for *E. coli* and 33.2% for *S. abony* after five-day storage.

Full inhibition of viable cells was detected in *C. albicans* 2 h after treatment of the wrapping paper with clary sage essential oil.

Unbleached cellulose wrapping paper started to inhibits the growth of *A. brasiliensis* (88.8%) 2 h after the addition of the clary sage essential oil.

The usage of recycled paper (Figure 4), 2 h after the addition of clary sage essential oil, led to the suppression of the *S. aureus* (100%) and *B. subtilis* (76%) growth. After 24 h-storage, its antibacterial action against *S. aureus* decreased to 89.2%. After five days, the effectiveness of *S. aureus* was 68.2%, while it was 71.7% against *B. subtilis*.

Recycled paper inhibited the growth of *E. coli* and *S. abony* bacteria by 100% and 71.3%, respectively, two hours after the treatment with clary sage essential oil. After 24 h-storage and 5 day-storage, its antimicrobial activity against *E. coli* decreased to 64.4%.

Treatment of recycled paper led to 100% inhibition of *C. albicans* yeast growth and 99.2% of *A. brasiliensis* mold. After 24 h, the effectiveness of the antimicrobial activity remained at the same level as both test microorganisms. After 5 days of storage, the antimicrobial effect was 99.7% for *C. albicans* and 81.9% for *A. brasiliensis*.

Previous studies showed that terpene essential oil components are effective on the biological activities on the common prominent pathogenic and spoilage food-related bacteria [14]. Our results for antimicrobial potential of clary sage essential oil are in agreement with the data found in the literature. Its high antimicrobial action could be probably due to the high content of oxygenated monoterpenes linalyl acetate and β-linalool.

Conclusion

1. The results obtained showed high fungicidal efficacy for the three types of packaging materials. The suppressive action against *C. albicans* during the investigated shelf life varied about 100%. A high antimicrobial efficiency against *A. brasiliensis* was found in the recycled paper (99.2% - 81.9%). The antimicrobial effectiveness in the bleached paper was found to be 83.9% and reduced to 52.6%, while the antimicrobial activity of unbleached paper reduces from 88.8% at the beginning of the experiment to 61.2% at the end of the storage period.
2. The bactericidal efficacy of the three types of packaging papers, coated with clary sage essential oil against the Gram-positive test microorganisms tested was found to be among 100% and 76% and reduced to 89.8% - 52.5% at the end of the five-day storage period. It was determined that the bactericidal effect of the tested packaging materials was lower than the Gram-negative bacterium *S. abony*.
3. Clary sage essential oil may be used in food packing as an antimicrobial agent in order to improve its quality and extend its shelf life.

Acknowledgments: The authors acknowledge the support of the Science Fund of the University of Ruse, Bulgaria (project 2019/BRz-01).

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