POSSIBILITIES FOR IMPARTING ANTIBACTERIAL PROPERTIES TO PAPERS

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ABSTRACT

Paper is widely used as a packaging material because it is biodegradable and completely safe for the environment. The use of antimicrobials as a coating on paper can improve its mechanical, optical, physical and antimicrobial properties. Antimicrobial packaging suppresses the development of pathogenic microorganisms in order to ensure the safety of the product, extend the shelf life and protect the quality of the product. Giving antibacterial properties to papers and cardboards expands their use in different areas and for different purposes. Antibacterial paper prevents the entry and development of harmful microorganisms such as bacteria, fungi, mould. Antimicrobials that occur naturally in nature have a number of advantages, as they are considered to pose less risk to the consumer. One of the modern directions in research is the inclusion of different types of active substances in the packaging material, which can give it additional functions, such as elimination of oxygen, moisture, ethylene, ethanol emission, antimicrobial activity and others. The present work aims to obtain plant extracts, their characterization and their surface application on paper. Extracts of Bulgarian herbs St. John's wort, cumin, rosemary, thyme and basil were obtained and characterized and studied. Plant extracts are applied by spraying on the surface of various papers. The antimicrobial effect of treated papers on gram-positive and gram-negative bacteria was studied. The results of the analysis of the obtained papers show the possibility of using plant extracts containing biologically active ingredients for the production of papers with barrier properties. The use of plant extracts is a promising and innovative solution.

Keywords: paper, antibacterial properties, plant extracts.

INTRODUCTION

Some plants possess antibacterial activity against a wide range of pathogenic bacteria. The antibacterial character may be due to bioactive compounds or to phenolic compounds of lignin. Various herbs are used in the pharmaceutical, cosmetic and textile industries [1]. A number of innovative applications have been explored [2 - 10]. Studies on the effect of plant extracts on paper are scarce. That is why, in our opinion, it is interesting to determine the phytochemical composition and biological activity of extracts from some Bulgarian herbs and their influence on different types of paper.

Antibacterial packaging materials are a form of active materials that slow down the development of microorganisms, extend the shelf life of products, improve their quality and safety. Of interest is the study of the possibility of including in the packaging papers plant extracts of herbs with active bio-ingredients such as rosemary, thyme, basil, cumin and St. John's wort, which give antimicrobial barrier properties to the paper [11 - 15]. Thyme is some perennial herb rich in various nutrients. The herb contains carvacrol and thymol. Carvacrol shows antifungal, insecticidal, antitoxic and antiparasitic activities. Thymol is a specific antimicrobial agent that acts in the fight against various types of pathogenic microorganisms - viruses, fungi, parasites, bacteria [11]. Rosemary, and in particular the flower

tops, contain antibacterial and antioxidant rosemary acid, as well as essential oils such as cineole, camphene, borneol, bornyl acetate, and α-pinene, which are known to have anti-inflammatory, antifungal and antiseptic properties. The herb is preferred by the pharmaceutical and cosmetic industries because of its antibacterial and anti-inflammatory properties [12, 13]. Basil is an annual herbaceous plant with a characteristic pleasant aroma. The flowering aboveground part is used. It contains about 1.5 % essential oil with a very diverse composition. Basil has antiseptic, antispasmodic, analgesic, anti-inflammatory and is also highly antibacterial [14]. Kim is a biennial to perennial herbaceous plant. It has a sharp aniseed aroma and taste. The aroma is of essential oils - mainly carvone and lemon. It contains significant amounts of nutrients, vitamins, minerals and antioxidants [15]. St. John's wort is a perennial herb. It has a variety of very useful properties. It has anti-inflammatory, antimicrobial and antiulcer effects. The herb has antiseptic and bactericidal properties [16]. Contains volatile substances that have a pronounced antibacterial effect.

The aim of this study was to obtain and test paper wrapping materials with plant extracts containing biologically active substances. The substances were obtained from widely found in Bulgarian mountains, herbs - St. John's Wort, Kim, Rosemary, Thyme and Basil. The resulting plant extracts of the different herbs were used for surface treatment of different packaging papers. Processed papers are microbiologically and mechanically tested to determine their properties and the capabilities to be used as an active packaging.

EXPERIMENTAL

Materials and methods

Raw materials

The following essential oil plants were used: thyme (*Thymus vulgaris* L.), basil (*Ocimum basilicum* L.), rosemary (*Rosmarinus officinalis* L.), St. John's wort (*Hypericum perforatum* L.), Cym (*Carum carvi* L.). They were purchased from the retail network. Before processing, the cumin fruits are ground in a laboratory mill.

A solvent of 70 % ethanol was used to prepare the liquid extracts. Static extractions were performed at a raw material ratio: solvent 1:10, temperature 60°C and duration 5 h. The separation of the raw material from the obtained extracts is by filtration through filter paper.

The extracts were analysed and the physical and chemical parameters were determined. The extracts were analysed and the physical and chemical parameters were determined: appearance, colour, odour and taste [17], refractive index (n_D^{20}) [17], relative density (d_{20}^{20}) [17], dry matter [17], tannins - by titration of the extract with 0,1 N $KMnO_4$ at the indigo carmine indicator [18].

Antimicrobial activity

Test microorganisms

To determine the antimicrobial action of the extracts of cumin, basil, thyme, rosemary and St. John's wort were used test cultures from National Bank for Industrial Microorganisms and Cell Cultures, Sofia: Gram-positive: *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Kocuria rhizophila* ATCC 9341; Gram-negative: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella abony* NTCC 6017; Yeast: *Saccharomyces cerevisiae* ATCC 9763, *Candida albicans* ATCC 10231; Mould fungi: *Aspergillus brasiliensis* ATCC 16404, *Fusarium moniliformae*. All used test microorganisms are deposited in the microbial cultural collection of the Department of Biotechnology and Food Technology at the University of Russe, Razgrad Branch.

Determination of antimicrobial activity by agar diffusion method

The experiments were performed on nutrient medium Soy casein agar (Biolife) - for bacteria and Saburo-dextrose agar (Biolife) for yeast and fungi. The experiments were performed in parallel with solvent controls, taking into account and correcting its action.

Determination of the antimicrobial action of paper treated with the studied extracts

Cellular suspensions of Gram-positive *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633 and Gram-negative *Salmonella abony* NTCC 6017 were used in the experiments. From the three types of paper treated with extracts of cumin, basil, thyme, rosemary and St. John's wort, squares measuring 5 x 5 cm were prepared. Under aseptic conditions, using sterile tweezers, place each square in a sterile petri dish. Using a sterile pipette, 0.1 mL of the prepared cell suspensions drop onto each square and carefully spread

on the surface of the paper, then placed in a thermostat at 35°C ± 2°C for 18 - 20 hours.

The following control samples were prepared: crude paper without bacterial suspension and crude paper with bacterial suspension. The samples were cultured in a thermostat at 35°C ± 2°C for 24 - 48 hours.

The effect of the extract-treated paper on the growth of the test micro-organisms was assessed by comparing the number of germinated bacteria from each suspension and the treated paper with that of the respective control samples (untreated paper with bacterial suspension).

Inhibition of bacterial growth is reported in this treatment, which shows 90 % reduction in the number of bacteria compared to that of the corresponding control sample [19, 20].

Determination of physical and mechanical properties of papers

Treated and unprocessed papers were tested to determine their physical and mechanical properties [21].

RESULTS AND DISCUSSION

Table 1 presents the results of organoleptic (colour, odour and taste) and physicochemical analyses of the extracts.

The results from the study of the antimicrobial activity of extracts from Cym (*Carum carvi* L.), Basil (*Ocimum basilicum* L.), Thyme (*Thymus vulgaris* L.), Rosemary (*Rosmarinus officinalis* L.) and St. John's wort (*Hypericum perforatum* L.) are given in Table 2.

The results presented in Table 2 show that the Gram-positive bacteria studied *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative *Salmonella abony* are sensitive to the action of rosemary extract. Yeast and moulds are not sensitive to this extract. The extracts of basil and St. John's wort have a weak antimicrobial effect against the studied Gram-positive bacteria and do not have one against the Gram-negative bacteria, yeast and mould fungi. Thyme extract has low antimicrobial activity against Gram-positive bacteria *Bacillus subtilis* and Gram-negative *Salmonella abony*. Cym extract has little effect on Gram-positive bacteria *Staphylococcus aureus, Bacillus subtilis* and Gram-negative *Salmonella abony*.

Three types of paper were used in the experiments: Offset Paper, Recycled and Packaging Paper. The results obtained are presented in Tables 3, 4 and 5.

Bleached offset paper treated with rosemary extract showed the greatest reduction in the number of viable microorganisms, 76.1 % in *Staphylococcus aureus*, 72 % in *Bacillus subtilis* and 84 % in *Salmonella abony*. Treatment with other extracts has little or no effect. The reduction in the number of viable microorganisms is between 0 and 55 %.

Treatment of recycled paper with Rosemary extract reduces the number of viable cells of *Staphylococcus aureus* by 74 %, *Bacillus subtilis* - 78.5 % and *Salmonella abony* - 70.3 %. Extracts of Gym, Thyme and St. John's Wort cause a reduction in the number of viable cells of *Bacillus subtilis* - 52.5 %, 50 % and 67.5 %, respectively.

Treatment of wrapping paper with rosemary extract

Table 1. Indicators of extracts of essential oil plants.

Extract	Color, smell and taste	Relative density, d_D^D	Refractive index, n_D^D	Dry substance, %	Tannins in the extract, %
Thyme	Dark green, specific smell and taste	0.8949 ± 0.002	1.3698 ± 0.004	2.30 ± 0.02	0.86 ± 0.03
Basil	Green-brown, specific smell and taste	0.8930 ± 0.001	1.3690 ± 0.001	1.97 ± 0.05	0.42 ± 0.02
Rosemary	Yellow-brown, specific smell and taste	0.8902 ± 0.005	1.3699 ± 0.003	1.77 ± 0.02	1.10 ± 0.02
St. John's Wort	Red-brown, specific smell and taste	0.8892 ± 0.002	1.3715 ± 0.001	2.78 ± 0. 03	0.86 ± 0.03
Cym	Yellow-brown, specific smell and taste	0.9097 ± 0.001	1.3702 ± 0.002	1.14 ± 0.03	0.10 ± 0.01

Table 2. Antimicrobial activity of extracts of Cym, Basil, Thyme, Rosemary and St. John's Wort.

Test microorganisms	Zone diameter (mm)				
	Cym	Basil	Thyme	Rosemary	St.John's Wort
Staphylococcus aureus ATCC 6538	9.0 ± 0.001	10.9 ± 0.003	0	17.6 ± 0.004	10.5 ± 0.001
Bacillus subtilis ATCC 6633	9.0 ± 0.003	12.1 ± 0.002	1.6 ± 0.001	17.3 ± 0.002	10.6 ± 0.002
Kocuria rhizophila ATCC 9341	0	13.0 ± 0.001	0	13.4 ± 0.001	11.3 ± 0.003
Escherichia coli ATCC 8739	0	0	0	0	0
Pseudomonas aeruginosa ATCC 9027	0	0	0	0	0
Salmonella abony NCTC 6017	9.0 ± 0.001	0	11.4 ± 0.001	16.5 ± 0.005	0
Candida albicans ATCC 10231	0	0	0	0	0
Saccharomyces cerevisiae	0	0	0	0	0
Aspergillus brasiliensis ATCC 16404	0	0	0	0	0
Fusarium moniliformae	0	0	0	0	0

Table 3. Influence of Offset paper treated with extracts of Cym, Basil, Rosemary, Thyme and St. John's Wort on the growth of test microorganisms

Used extract	Test microorganisms	Total number of viable microorganisms (cfu mL^{-1})	Processing efficiency (%)
-	-	90	-
-	*Staphylococcus aureus*	$3.8x10^5$	-
-	*Bacillus subtilis*	$3x10^5$	-
-	*Salmonella abony*	$6x10^5$	-
Cym	-	174	-
	Staphylococcus aureus	$3.3x10^5$	13.4
	Bacillus subtilis	$2.1x10^5$	30.0
	Salmonella abony	$2.7x10^5$	55.0
Basil	-	0	-
	Staphylococcus aureus	$3.6x10^5$	5.3
	Bacillus subtilis	$2.4x10^5$	20.0
	Salmonella abony	$4.4x10^5$	26.7
Rosemary	-	4	-
	Staphylococcus aureus	$9.1x10^4$	76.1
	Bacillus subtilis	$8.4x10^4$	72.0
	Salmonella abony	$9.6x10^4$	84.0
Thyme	-	18	-
	Staphylococcus aureus	$3.4x10^5$	10.5
	Bacillus subtilis	$2.9x10^5$	3.3
	Salmonella abony	$4.5x10^5$	25.0
St. John's wort	-	8	-
	Staphylococcus aureus	$3.8x10^5$	0
	Bacillus subtilis	$2.7x10^5$	10.0
	Salmonella abony	$3.7x10^5$	38.3

Table 4. Influence of recycled paper treated with extracts of Gym, Basil, Rosemary, Thyme and St. John's Wort on the growth of test microorganisms.

Used extract	Test microorganisms	Total number of viable microorganisms (cfu mL^{-1})	Processing efficiency (%)
-	-	210	-
-	*Staphylococcus aureus*	$2.3x10^5$	-
-	*Bacillus subtilis*	$4x10^5$	-
-	*Salmonella abony*	$3.1x10^5$	-
Cym	-	140	-
	Staphylococcus aureus	$1.6x10^5$	30.4
	Bacillus subtilis	$1.9x10^5$	52.5
	Salmonella abony	$2.6x10^5$	16.1
Basil	-	200	-
	Staphylococcus aureus	$1.7x10^5$	26.1
	Bacillus subtilis	$3.1x10^5$	22.5
	Salmonella abony	$2.1x10^5$	32.3
Rosemary	-	60	-
	Staphylococcus aureus	$6x10^4$	74
	Bacillus subtilis	$8.6x10^4$	78.5
	Salmonella abony	$9.2x10^4$	70.3
Thyme	-	230	-
	Staphylococcus aureus	$2.2x10^5$	4.4
	Bacillus subtilis	$2x10^5$	50
	Salmonella abony	$1.9x10^5$	38.7
St. John's wort	-	190	-
	Staphylococcus aureus	$2.1x10^5$	8.7
	Bacillus subtilis	$1.3x10^5$	67.5
	Salmonella abony	$1.5x10^5$	51.6

Table 5. Influence of Gin, Basil, Rosemary, Thyme and St. John's Wort extracts on wrapping paper on the growth of test microorganisms.

Used extract	Test microorganisms	Total number of viable microorganisms (cfu mL^{-1})	Processing efficiency (%)
-	-	110	-
-	*Staphylococcus aureus*	$2x10^5$	-
-	*Bacillus subtilis*	$5x10^5$	-
-	*Salmonella abony*	$4.5x10^5$	-
Cym	-	804	-
	Staphylococcus aureus	$1.7x10^5$	15
	Bacillus subtilis	$3x10^5$	50
	Salmonella abony	$2.5x10^5$	44.5
Basil	-	28	-
	Staphylococcus aureus	$1.3x10^5$	50
	Bacillus subtilis	$4.3x10^5$	14
	Salmonella abony	$4.1x10^5$	8.9
Rosemary	-	0	-
	Staphylococcus aureus	$4x10^4$	80
	Bacillus subtilis	$1.7x10^5$	66
	Salmonella abony	$3.6x10^5$	20

Table 5. Influence of Gin, Basil, Rosemary, Thyme and St. John's Wort extracts on wrapping paper on the growth of test microorganisms. - *continued*

Used extract	Test microorganisms	Total number of viable microorganisms (cfu mL^{-1})	Processing efficiency (%)
Thyme	-	123	-
	Staphylococcus aureus	$4x10^5$	50
	Bacillus subtilis	$3.2x10^5$	36
	Salmonella abony	$4.1x10^5$	8.9
St. John's wort	-	10	-
	Staphylococcus aureus	$1.8x10^5$	10
	Bacillus subtilis	$4.2x10^5$	16
	Salmonella abony	$3.8x10^5$	15.6

caused a reduction in the number of viable cells of *Staphylococcus aureus* by 80%, *Bacillus subtilis* - 66 % and *Salmonella abony* - 20 %.

The physico-mechanical parameters of the three types of packaging papers treated with plant extracts were studied - from bleached cellulose, from unbleached cellulose and from recycled paper. The results of the analyses for the breaking force in the longitudinal and transverse directions are presented in Figs. 1 - 3. The figures show that the surface treatment with plant extracts of the paper changes little, but there is no significant reduction in the strength of the paper samples.

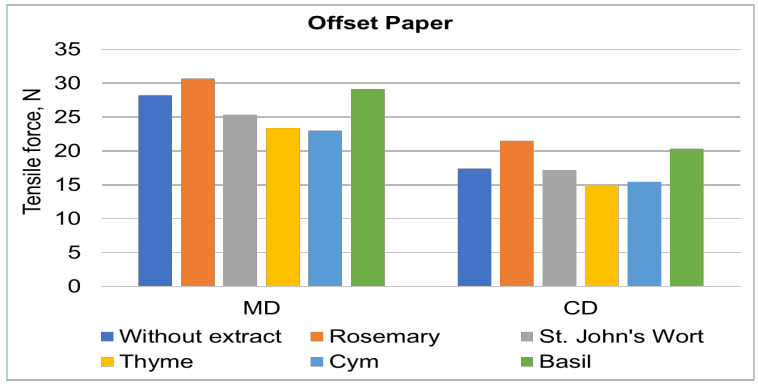

Fig. 1. Tensile force change of offset paper samples treated with plant extracts.

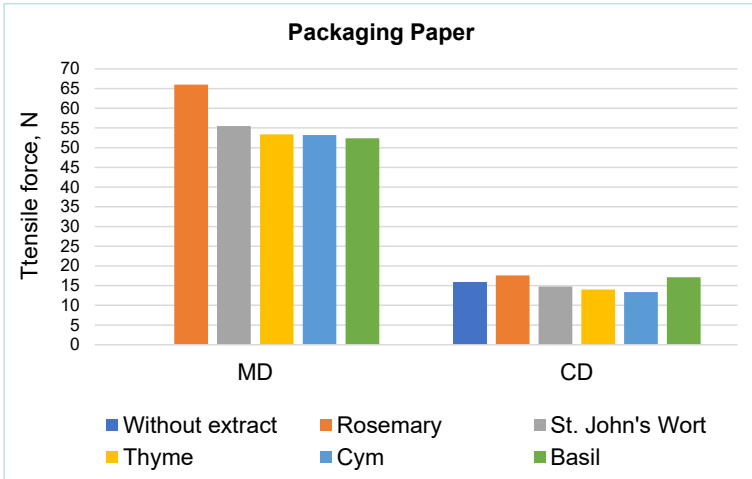

Fig. 2. Tensile force change of packaging paper samples treated with plant extracts.

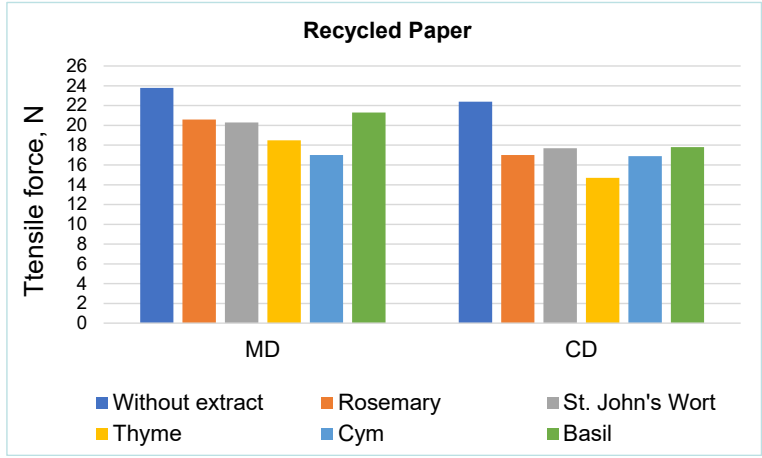

Fig. 3. Tensile force change of recycled paper samples treated with plant extracts

CONCLUSIONS

Plant extracts of Bulgarian herbs - cumin, basil, thyme, rosemary and St. John's wort were obtained, studied and analysed. The antimicrobial activity of the extracts was studied. Three types of paper were processed and analysed with them - bleached cellulose, unbleached cellulose packaging paper and recycled paper. The antimicrobial effect on three types of paper with the studied extracts was determined. The results show that treatment with rosemary extract has the strongest effect on all three types of paper. The physico-mechanical properties do not decrease significantly and are practically preserved. This gives rise to plant extracts of rosemary, cumin, basil and thyme to be recommended for processing various types of paper and making active packaging.

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