Antibacterial activity of biopolymer composite materials obtained from pumpkin oil cake and winter savory or basil essential oil against various pathogenic bacteria

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Summary

Pumpkin oil cake, a by-product obtained after oil extraction from pumpkin seed, possesses good film-forming properties, as well as the ability to be used as a matrix for various compounds in order to obtain active biopolymer materials. Use of essential oils, as active components with a pronounced activity, can be a good alternative to synthetic chemical compounds. In the present work, composite pumpkin oil cake-based films with winter savory or basil essential oil were developed and their antibacterial activity against two Gram-negative bacteria (*E. coli*, *S.* Enteritidis) and three Gram-positive bacteria (*L. monocytogenes*, *Staph. aureus* and *B. cereus*) was analysed. Gas chromatographic-mass spectrometric analysis confirmed the main phenolic compounds to be carvacrol in winter savory essential oil and linalool in basil essential oil. Pumpkin oil cake film without essential oils did not show antibacterial activity against any of the tested bacteria, while films with basil essential oil showed some antibacterial activity against *L. monocytogenes* and *B. cereus*. However, films with winter savory essential oil showed antibacterial activity in all added concentrations against all 5 bacteria. Addition of winter savory and basil essential oils into pumpkin oil cake-based film enhanced its antibacterial activity, demonstrating its possible application as an active composite material.

Keywords

biopolymer composite material; pumpkin oil cake; winter savory essential oil; basil essential oil; pathogenic bacteria; antibacterial activity

For decades, traditional food packaging has been used to provide passive protection of products against various external conditions that affect its quality and shelf life. A new alternative method is an active packaging system, which is defined as a material that interacts with the product and releases or absorbs components in or from food, in order to maintain the quality of the packed product or prolong its shelf life. The active compounds may be directly incorporated into the packaging material and, in this way, slow or controlled release of the active compounds from the material into the headspace of packaging, or directly on product surface, can be achieved during its shelf life [1]. This is of particular importance in the case of microbiological food contamination because microbial growth on food surfaces is considered a major cause of food spoilage and food poisoning caused by contaminated food [2]. For years, a huge number of large outbreaks of food poisoning around the world were caused by various Gram-negative or Gram-positive bacteria in food, some diseases caused by them, such as human listeriosis, being characterized by a high mortality rate [3].

Biopolymer materials are obtained from natural sources and often decomposed naturally in the environment. They represent an interesting alternative to synthetic materials in the packaging industry, regarding ecological aspects. They are

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usually synthetized from proteins, polysaccharides or lipids. Food industry produces large amounts of waste or by-products that can be rich in these components. That is why agro-industrial by-products could be attractive renewable substrates for production of biopolymer materials, which could contribute to the reduction of organic waste.

Pumpkin seed (*Cucurbita pepo* L.) is considered an important oil crop in Serbia, and is widely used for production of edible oil [4]. After oil production, the main by-product obtained after oil extraction from seeds is a defatted pumpkin oil cake (PuOC), which is rich in proteins (accounting for approximately 63 %), carbohydrates, oils, crude fibres and other components [5]. Because of its chemical composition, PuOC represents a raw material convenient for obtaining biopolymer composite films [5] as well as active composite films with various additives. Optimal properties, in particular good barrier properties to gases of PuOC-based material, were already reported in the literature [5]. Today, research is devoted to replacing synthetic chemical additives with the natural ones, so use of essential oils, as an alternative to synthetic chemical additives has been studied [1, 6]. Essential oils exhibit antimicrobial and antioxidant properties mostly because of the presence of phenolic compounds [1, 7]. According to the European Commission [8], an active compound can be incorporated into food packaging in order to be released during storage or to absorb substances from the food, if it is included in positive lists of authorized substances applicable to food. Several essential oils that can be applied to food packaging have been approved by the Food and Drug Administration in USA [9]. One of major obstacles for direct addition of essential oils to food is their strong flavour, which may restrict their applications [1]. However, their incorporation into biopolymer packaging materials can overcome this and other problems [6]. Furthermore, it has been stated that, in comparison with direct application, smaller amounts of active agents would be needed when biopolymer materials are used as carriers [6].

Aromatic plant *Satureja montana* L., commonly known as winter savory, belongs to the Lamiaceae family and is widely spread in the Balkan region, including Serbia. The most significant and dominant phenolic compound of *Satureja montana* L. essential oil is usually carvacrol, which is responsible for its biological effects [7]. Sweet basil (*Ocimum basilicum* L.) also belongs to the Lamiaceae family. In the basil essential oil, linalool is usually the major active component, exhibiting various biological activities [10]. However, linalool and carvacrol, as well as other phenolic compounds, are unstable, volatile and readily oxidizable [11]. A way of their protection could be their incorporation into polymer materials.

Despite the well-known antimicrobial activity of winter savory [12–14] and basil [15, 16] essential oils, their potential in development of active biopolymer packaging materials is still insufficiently explored. The aim of this work was to produce composite PuOC-based active packaging materials with winter savory or basil essential oil, and to examine their antibacterial activity against two Gram-negative (*E. coli* and *S.* Enteritidis) and three Gram-positive bacteria (*L. monocytogenes*, *Staph. aureus* and *B. cereus*) that are common pathogenic bacteria that cause food spoilage, as well as foodborne illness resulting from consumption of contaminated food. Gas chromatographic-mass spectrometric (GC-MS) analysis was used to identify the main and the most abundant compounds in winter savory and basil essential oils that could probably be responsible for their activity.

MATERIALS AND METHODS

The grounded hull-less pumpkin oil cake was kindly provided by Linum (Čonoplja, Serbia). Winter savory (*Satureja montana* L.) essential oil and basil (*Ocimum basilicum* L.) essential oil were kindly supplied by Institute of Field and Vegetable Crops (Novi Sad, Serbia). The plants were collected in Bački Petrovac (Serbia) and the essential oils were obtained by steam distillation at the Institute of Field and Vegetable Crops. Reagents used in this study were of analytical grade.

Preparations of samples

Film-forming suspension based on PuOC was prepared by the casting method described by Bulut et al. [17]. Active films with essential oils were obtained after incorporation of essential oils in concentrations of 30 $ml \cdot l^{-1}$, 40 $ml \cdot l^{-1}$ and 50 $ml \cdot l^{-1}$ of the obtained filtrate. Emulsions were homogenized with Ultra Turrax (IKA-Werke, Staufen, Germany) for 2 min, 333.33 Hz, 2 times, and casted onto Teflon-coated Petri dishes and dried for 3 days at (23 ± 2) °C, (50 ± 5) % relative humidity. A film without addition of essential oils was used as a control. To measure antibacterial activity of free essential oils, they were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, Missouri, USA) in defined concentrations. Obtained samples were labelled and are presented in Tab. 1.

Tab. 1. Designation and description of tested samples.

Sample	Composition			
	PuOC film/ EO	Basil EO [ml·l^{-1}]	Winter savory EO [ml·l^{-1}]	DMSO [ml]
Control	PuOC film	0	0	0
PW3	PuOC film	0	30	0
EW3	EO	0	30	1
PW4	PuOC film	0	40	0
EW4	EO	0	40	1
PW5	PuOC film	0	50	0
EW5	EO	0	50	1
PB3	PuOC film	30	0	0
EB3	EO	30	0	1
PB4	PuOC film	40	0	0
EB4	EO	40	0	1
PB5	PuOC film	50	0	0
EB5	EO	50	0	1

PuOC – pumpkin oil cake, EO – essential oil, DMSO – dimethyl sulfoxide.

Gas chromatography-mass spectrometry

GC-MS analysis is the most commonly used method for the determination of essential oil components. The composition of tested essential oils was analysed with a gas chromatograph GC 7890B (Agilent Technologies, Santa Clara, California, USA) coupled to mass spectrometer MS 5977A (Agilent Technologies) using a HP-5MS capillary column (30 m × 0.25 mm 5% phenyl-methylpolysiloxane, film thickness 0.25 μm; Agilent Technologies). The identity of the components of the essential oils was assigned by comparison of their retention indices and mass spectra with literature data [18, 19] and the mass spectra databases Wiley 10th (John Wiley and Sons, Hoboken, New Jersey, USA) and NIST 2011 MS Library (National Institute of Standards and Technology, Gaithersburg, Maryland, USA). Retention indices (*RI*) were determined relative to the retention times of a series of *n*-alkanes by linear interpolation. Relative contents of the components were calculated by the area normalization method, without considering response factors. The component percentages were calculated as mean values from duplicate GC–MS analyses.

Antibacterial activity

The antibacterial activity of the samples against five bacterial strains was examined by the disk diffusion method. The bacterial strains in a KWIK-STIK format were obtained from Microbiologics (St. Cloud, Minnesota, USA) and comprised *Escherichia coli* ATCC 25922, *Salmonella enterica* subsp. *enterica* serovar Enteritidis (group D) ATCC 13076), *Listeria monocytogenes* ATCC 19111, *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 25923. The bacterial strains were cultured at 37 °C for 24 h on nutrient agar (HiMedia, Mumbai, India). After incubation, the microbial biomass of the culture was transferred to 10 ml of sterile physiological saline solution (8.5 g·l^{-1} NaCl) to reach the suspension concentration of 10^8 CFU·ml^{-1}. Concentration of the bacterial suspension was determined with McFarland nefelometer (SIA Biosan, Riga, Latvia). Suspensions were decimally diluted in physiological saline solution to obtain the suspension of 10^6 CFU·ml^{-1}, which was used to inoculate Petri dishes with Mueller-Hinton agar (Merck, Darmstadt, Germany). To evaluate the antibacterial activity of active films, films were sterilized under UV lamp, cut into a circular shape (7 mm in diameter) using a sterile cork borer, and placed on the surface of Mueller-Hinton agar (one film per Petri dish in three repetitions). Prepared Petri dishes were sealed using parafilm and incubated at 37 °C for 24 h. The diameters of inhibition zones were measured in millimeters.

Statistical analysis

Statistical analysis was carried out using Statistica 13.5.0.17 (StatSoft, Tulsa, Oklahoma, USA). Data were presented as mean values with standard deviation. The variance analysis (ANOVA) was performed with a confidence interval of 95 % ($p < 0.05$) and the means obtained were compared by the Tukey's test.

RESULTS AND DISCUSSION

Gas chromatography-mass spectrometry

GC-MS results on the composition of *Satureja montana* essential oil are presented in Tab. 2. Thirty-five compounds were identified corresponding to 98.2 % of the total composition. The most abundant compounds were carvacrol (47.7 %), *p*-cymene (27.4 %) and γ-terpinene (7.4 %). The share of all other components was lower than 5 %. These results are in accordance with previous studies, which reported carvacrol [12, 20–22], *p*-cymene [20–22] and γ-terpinene [21, 22] as the main and the most abundant components of *Satureja montana* essential oil. Literature data suggested that composition of *Satureja montana* essential oil and other *Satureja* species differed and depended mostly on geographic location, season, seasonal variations or environmental conditions under which the plant grew and on

drying, as well as on genetic characteristics of the plant and the method of obtaining essential oil [12, 21, 22].

In *Ocimum basilicum* essential oil, 51 compounds were identified, corresponding to 88.6 % of the total composition (Tab. 3). The major compound was found to be linalool (43.7 %), while the share of all other identified components was quite low (less than 5% each). These results are in accordance with FILIP et al. [23] who also reported linalool as a major compound with the highest percentage (49.8 %) in the *Ocimum basilicum* essential oil also grown in Serbia.

Tab. 2. Chemical composition of *Satureja montana* L. essential oil.

Retention time [min]	Compound	Peak area [%]
4.331	α-Thujene	0.1
4.492	α-Pinene	0.5
4.798	Camphene	0.2
5.354	1-Octen-3-ol	1.0
5.650	β-Myrcene	0.4
6.018	α-Phellandrene	0.1
6.333	α-Terpinene	1.1
6.584	*p*-Cymene	27.4
6.656	Limonene	0.5
6.728	1,8-Cineole	0.3
7.473	γ-Terpinene	7.4
8.604	Linalool	1.2
10.659	endo-Borneol	1.5
10.857	DL-Menthol	0.2
11.027	Terpinen-4-ol	0.9
11.252	*p*-Cymenol	0.1
11.440	α-Terpineol	0.2
11.665	Estragole	0.1
13.262	Carvone	0.1
14.663	Thymol	0.2
15.138	Carvacrol	47.7
17.365	Copaene	0.2
17.652	β-Bourbonene	0.1
18.729	Caryophyllene	1.8
19.752	α-Humulene	0.1
20.452	γ-Muurolene	0.3
21.009	Zingiberene	0.2
21.162	α-Muurolene	0.1
21.413	β-Bisabolene	1.1
21.574	γ-Cadinene	0.3
21.844	δ-Cadinene	0.6
22.409	α-Calacorene	0.1
22.723	Paeonal	1.1
23.424	Spathulenol	0.1
23.594	Caryophylene oxide	1.0
Percentage of identified compounds		98.2
Percentage of not identified compounds		1.9

Tab. 3. Chemical composition of *Ocimum basilicum* L. essential oil.

Retention time [min]	Compound	Peak area [%]
6.521	*p*-Cymene	0.2
6.638	Limonene	0.1
6.719	1,8-Cineole	1.9
7.850	Linalool oxide	0.3
8.693	Linalool	43.7
8.864	α-Thujone	0.7
9.160	β-Thujone	0.3
10.013	Camphor	0.8
10.291	Menthone	0.2
10.650	endo-Borneol	0.3
10.857	DL-Menthol	0.6
11.027	Terpinen-4-ol	0.3
11.440	α-Terpineol	0.5
11.683	Estragole	3.9
13.451	Geraniol	0.7
14.492	Borneol acetate	1.3
16.521	α-Cubebene	0.2
16.736	Eugenol	0.8
17.356	Copaene	0.4
17.580	Geranyl acetate	0.4
17.643	β-Bourbonene	1.0
17.867	β-Elemene	2.2
18.235	Methyleugenol	0.1
18.711	Caryophyllene	1.0
19.106	Calarene	1.1
19.205	α-Bergamotene	3.0
19.303	α-Guaiene	1.0
19.761	α-Humulene	2.1
19.977	Aromadendrene	0.2
20.040	*epi*-Bicyclosesquiphellandrene	0.4
20.452	γ-Muurolene	0.3
20.596	Germacrene	0.4
20.749	β-Selinene	0.6
21.009	Eudesma-3,11-diene	0.4
21.117	Pentadecane	0.4
21.341	α-Bulnesene	1.4
21.583	γ-Cadinene	4.1
21.835	Calamenene	0.9
23.002	Nerolidol	0.4
23.127	β-Maaliene	2.2
23.424	Spathulenol	1.1
23.594	Caryophylene oxide	0.9
23.837	Globulol	1.8
24.016	Hexadecane	0.4
24.348	Humulene epoxide	1.7
25.470	β-Eudesmol	0.3
26.781	Heptadecane	0.4
29.420	Octadecane	0.3
30.578	Perhydrofarnesyl acetone	0.2
31.942	Nonadecane	0.1
35.658	Epimanool	0.5
Percentage of identified compounds		88.6
Percentage of not identified compounds		11.4

Jelačić et al. [24] analysed chemical composition of basil essential oil isolated from herbs of ten basil species, also traditionally grown on the territory of Serbia, and reported that the dominant compound in all ten isolated essential oils was linalool, share of which varied from 51.5 % to 74.7 %. According to the literature data [23, 24], composition of basil essential oils differs mostly due to geographic location, environmental and genetic factors, and for *Ocimum basilicum* L. that was grown in the territory of Serbia, the dominant component of essential oil is usually linalool.

Antibacterial activity

The disk diffusion method primarily simulates materials that are in direct contact with products and tests the possibility of release of the active compounds from material into the product, for the purpose of reducing, delaying or preventing microbial contamination. Ponce et al. [25] established classification of sensitivity of bacteria to antibacterial compounds by the diameter of the inhibition zone (not sensitive – for diameters less than 8 mm, sensitive – for diameters from 9 mm to 14 mm, very sensitive – for diameters from 15 mm to 19 mm and extremely sensitive – for diameters larger than 20 mm).

Tab. 4. represents the antibacterial activity of samples against two Gram-negative bacteria, *E. coli*, and *S.* Enteritidis, and three Gram-positive bacteria, *L. monocytogenes, Staph. aureus* and *B. cereus*. According to the results, the control film did not show antibacterial activity against any of the tested bacteria. Films with basil essential oil as well as pure basil essential oil showed poor antibacterial activity against the tested bacteria, where diameters of zone of inhibition ranged between 7 mm and 9 mm. The only samples that showed significantly greater ($p < 0.05$) diameters of inhibition zone were PuOC-based films with basil essential oil against *L. monocytogenes*, where the diameter of the inhibition zone ranged between 11 mm (PB3, PB4) and 13 mm (PB5). The antibacterial activity of basil essential oil against various bacteria was confirmed [15, 16], attributing most commonly to linalool as the main component. However, basil essential oil used in this study, despite the high content of linalool, showed poor antibacterial activity, which was further reduced by its incorporation into PuOC-based film. The reason may be the higher content of other components reported in the literature, such as methyl chavicol (estragole) [15], as well as *epi*-α-cadinol and α-bergamotene [16], which could contribute to greater activity and synergistic effects with linalool. Share of this compounds in the basil essential oil used in this research was lower than 5 %.

Free winter savory essential oil showed antibacterial activity against all tested bacteria. The most sensitive was *B. cereus*, especially to EW5, with an inhibition zone of 23 mm. Antimicrobial activity of winter savory essential oil against various bacteria, such as *Staphylococcus* spp., *E. coli*, *L. monocytogenes* or *S.* Typhimurium, was previously reported [12–14].

Previous studies described the antimicrobial activity of carvacrol, the major and the most abundant component of *Satureja montana* essential oil

Tab. 4. Antibacterial activity of biopolymer films and essential oils.

Sample	Antibacterial activity of samples [mm]				
	E. coli	*S.* Enteritidis	*L. monocytogenes*	*Staph. aureus*	*B. cereus*
Control	ND	ND	ND	ND	ND
PW3	10.0 ± 0.0^{aB}	11.0 ± 1.0^{abAB}	12.0 ± 0.0^{bD}	11.0 ± 1.0^{abCD}	12.0 ± 0.0^{bB}
EW3	9.5 ± 0.5^{aB}	10.5 ± 0.5^{aAB}	ND	10.0 ± 0.0^{aCD}	14.5 ± 0.5^{bC}
PW4	12.5 ± 0.5^{aC}	13.0 ± 0.0^{aB}	14.0 ± 0.0^{aF}	14.0 ± 1.0^{aE}	17.0 ± 1.0^{bD}
EW4	10.0 ± 0.0^{abB}	10.5 ± 0.5^{bAB}	9.0 ± 0.0^{aA}	9.5 ± 0.5^{abBC}	14.0 ± 1.0^{cC}
PW5	13.0 ± 0.0^{aC}	22.0 ± 3.0^{cC}	13.0 ± 0.0^{aE}	17.5 ± 1.5^{bF}	18.5 ± 0.5^{bcD}
EW5	9.5 ± 0.5^{aB}	13.0 ± 0.0^{cB}	10.0 ± 0.0^{aB}	11.5 ± 0.5^{bD}	23.0 ± 0.0^{dE}
PB3	ND	ND	11.0 ± 1.0^{C}	ND	ND
EB3	8.0 ± 0.0^{abA}	9.0 ± 0.0^{bA}	ND	7.0 ± 0.0^{aA}	8.0 ± 1.0^{abA}
PB4	ND	ND	11.0 ± 0.0^{C}	ND	ND
EB4	8.5 ± 0.5^{aA}	8.5 ± 0.5^{aA}	ND	7.5 ± 0.5^{aA}	8.5 ± 0.5^{aA}
PB5	ND	ND	13.0 ± 0.0^{bE}	ND	9.0 ± 0.0^{aA}
EB5	8.5 ± 0.5^{abA}	9.0 ± 0.0^{bA}	ND	8.0 ± 0.0^{aAB}	8.0 ± 0.0^{aA}

Data are mean values ± standard deviation. Different lower-case letters indicate significant differences among samples in rows ($p < 0.05$); different upper-case letters indicate significant differences among samples in columns ($p < 0.05$).
ND – not detected.

analyzed in this study, against various bacteria [26, 27]. It was suggested that the main reasons for high activity of carvacrol were its high hydrophobicity and the presence of a free hydroxyl group that was found to be essential for antimicrobial activity [28].

Essential oils, similar to other lipophilic compounds, possess a high affinity to cell membranes. The mechanism of antimicrobial activity is related to its ability to penetrate the cell wall of a bacterium and attack the phospholipid bilayer of cell membranes [29]. Phenolic compounds can disintegrate the outer membrane of gram-negative bacteria, and thus increase the cytoplasmic membrane permeability [30]. Burt [30] reported that carvacrol forms channels through the bacterial membrane by pushing apart the fatty acid chains of the phospholipids, allowing ions to leave the cytoplasm and increasing permeability of the cytoplasmic membrane to ATP, causing cell death.

According to the results, active PuOC-based films with winter savory essential oil showed antibacterial activity against all tested bacteria, which significantly increased with increasing essential oil's concentrations ($p < 0.05$). The greatest inhibitory zone was observed for PW5 against *S.* Enteritidis, which was extremely sensitive to this film, with an inhibitory zone of 22 mm. However, it can be considered that a very sensitive bacterium was also *B. cereus* due to high values of inhibitory zones, compared to other bacteria, for all concentrations of the oil. The least sensitive bacteria were *E. coli* and *L. monocytogenes*, which were sensitive to all films, with inhibitory zones in the range from 10 mm to 14 mm.

Based on the obtained results, PuOC-based films with winter savory essential oil showed higher antibacterial activity compared to films with basil essential oil. Martucci et al. [31] analysed bioactivity of active films with oregano and lavender essential oils, and the obtained results showed that films with oregano essential oil had slightly higher antibacterial activity. This was attributed to the stronger effect of carvacrol (the main component of oregano essential oil) compared to linalool (the main component of lavender essential oil). These results can be compared with results obtained in this study, taking into account that those are also the main components present in essential oils tested in this work.

When comparing antibacterial activity of free winter savory essential oil and active films with the same oil, interesting situation could be observed. All active films showed higher inhibitory zone compared to the same concentrations of free essential oil, especially with higher concentrations of added essential oil (PW4 and PW5), except in the case of *B. cereus*. Some literature data showed that essential oils encapsulated into biopolymer matrix showed higher antibacterial or antifungal activity compared to the respective free oil [32, 33], suggesting that encapsulation of essential oils into biopolymers could preserve them from degradation and/or evaporation, and protect them against the environmental conditions as well as enable their gradual release. Taking into account that biopolymer films are also considered as a suitable matrix or medium for various additives, it can be assumed that they also could protect active compounds from various environmental conditions.

Although no greater inhibitory zones were observed at films compared to free oil in the case of *B. cereus*, a phenomenon of double zones for PW4 and PW5 against *B. cereus* was observed. The first zone of inhibition was clear and complete inhibition without growth of bacteria was obtained. The second zone with a reduced bacterial growth, which surrounded the clear zone of inhibition, was the zone to which only a fraction of active compounds were able to diffuse from the biopolymer films, but this amount was not sufficient to cause complete inhibition. This could be attributed to interactions between polyphenols and film matrices, which may interfere and affect the diffusion of phenolics from the films into agar during time, as hypothesized by Pol et al. [34]. Those authors observed a reaction between carvacrol and proteins, which limited the antibacterial activity against *B. cereus*.

According to the obtained results, active materials based on PuOC and essential oils might have potential applications for packaging various food products, in particular products where an oxygen barrier is of great importance, such as edible oils or fatty products including meat and cheese, where the addition of essential oils need not have a negative sensory influence. On the contrary, essential oils may contribute positively to sensory properties of these products, prolonging their shelf life at the same time. The active material is provided to be in direct contact with product, in order to allow the gradual release of active components from the material to the product surface and inhibit the growth of microorganisms, primarily on the product surface. However, Cao-Hoang et al. [35] showed that an active compound may diffuse also to other parts of the product, i.e. to the internal parts of the food product, depending mostly on desorption of the active component from the film and its diffusion through the food matrix.

CONCLUSIONS

GC-MS analysis demonstrated the presence of the main phenolic compounds carvacrol and linalool in *Satureja montana* L. and *Ocimum basilicum* L. essential oils, respectively. Those phenolic compounds, known for their biological activity, may have contributed to the antibacterial activity of PuOC-based film after their incorporation. Films with basil essential oil showed some antibacterial activity only against *L. monocytogenes* and *B. cereus*. On contrary, incorporation of winter savory essential oil into PuOC-based film led to active films with good antibacterial properties against both Gram-negative bacteria (*E. coli* and *S.* Enteritidis) and Gram-positive bacteria (*L. monocytogenes*, *Staph. aureus* and *B. cereus*). Biopolymer films based on PuOC and winter savory essential oil, according to the obtained results, may have a potential application as active materials for packaging food products, in order to delay food contamination with Gram-positive and Gram-negative bacteria. Further research should include experiments with food products, where the influence of the of PuOC-based material with essential oils on the preservation and shelf life of the product, as well as on its sensory influence would be examined.

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