THE INFLUENCE OF ESSENTIAL OILS ON THE PROPERTIES OF BIOPOLYMER FILMS BASED ON WILD FLAX (*CAMELINA SATIVA* L.) UTICAJ ETARSKIH ULJA NA OSOBINE BIOPOLIMERNIH FILMOVA NA BAZI DIVLJEG LANA (*CAMELINA SATIVA* L.)

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ABSTRACT

The aim of this work was the activation of biopolymer material based on wild flax cake (Camelina Sativa) with eucalyptus and rosemary essential oils added in different concentrations (0.5%, 1% and 2%). The wild flax cake, left over after the cold pressing of the oil, was used to obtain biopolymer films. To obtain active packaging, essential oils were added to this biopolymer material and the influence of the addition of essential oils on the physico-chemical, mechanical, barrier and biological properties of biopolymer films based on wild flax cake was examined. The obtained results showed the most significant influence on water vapor permeability. The control sample had a water vapor permeability value of 5.43 g/m²h, while in the sample with 2% added eucalyptus essential oil, this value was 3.14 g/m²h. Antioxidant activity was also confirmed in the control sample without added essential oils (60.10%), but with the addition of 2% eucalyptus essential oil, this value increased to 78.54%. Eucalyptus essential oil proved to be more effective than rosemary oil on the investigated properties of the films. The addition of essential oils to biopolymer films broadens the spectrum of functional properties, including the improvement of mechanical, barrier and biological properties.

Keywords: Camelina sativa, essential oils, biopolymer films

REZIME

Poslednjih decenija naučna istraživanja su fokusirana na oblast biopolimernih materijala zbog njihovih brojnih prednosti i činjenice da se mogu koristiti kao dodatak ili potpuna zamena komercijalnih polimernih materijala. Ipak, zbog svoje jake hidrofilnosti, biopolimerni materijali imaju slabe barijerne osobine prema vodenoj pari i umerene mehaničke osobine, što je glavno ograničenje za njihovu široku primenu. Optimizacija svojstava predstavlja izazov u oblasti biopolimernih materijala, odnosno u oblasti aktivne ambalaže. Cilj ovog rada je aktivacija biopolimernog materijala na bazi pogače divljeg lana (Camelina sativa) etarskim uljima eukaliptusa i ruzmarina dodatim u različitim koncentracijama (0,5%, 1% i 2%). Pogača divljeg lana, zaostala nakon hladnog ceđenja ulja, iskorišćena je za dobijanje biopolimernih filmova. U cilju dobijanja aktivne ambalaže, etarska ulja su dodata u ovaj biopolimerni material i ispitan je uticaj dodatka essencijalnih ulja na fizičko-hemijske, mehaničke, barijerne i biološke osobine biopolimernih filmova na bazi pogače divljeg lana. Dobijeni rezultati su pokazali najznačajniji uticaj na propustljivost vodene pare. Kontrolni uzorak imao je vrednost propustljivosti vodene pare 5,43 g/m2h, dok je kod uzorka sa 2% dodatog etarskog ulja eukaliptusa ta vrednost iznosila 3,14 g/m2h. Antioksidativna aktivnost je potvrđena i kod kontrolnog uzorka bez dodatih etarskih ulja (60,10%), ali je dodatkom 2% etarskog ulja eukalipusa ova vrednost povećana na 78,54%. Etarsko ulje eukaliptusa se pokazalo efektnijim od ulja ruzmarina na ispitane osobine filmova. Dodavanje etarskih ulja biopolimernim filmovima proširuje spektar funkcionalnih svojstava, uključujući unapređenje mehaničkih, barijernih i bioloških osobina. Dalja istraživanja potrebno je usmeriti ka kompatibilnosti, stabilnosti i prihvatljivosti za potrošače.

Ključne reči: Camelina sativa, etarska ulja, biopolimerni filmovi

INTRODUCTION

In recent years, the quest for sustainable and environmentally friendly materials has led to a surge of interest in biopolymers derived from renewable resources (Christina et al., 2023). One of the directions of expansion of raw materials for obtaining biopolymers is the valorization of agro-industrial waste, which has been intensively studied (Klai et al., 2021). There are many sources of waste: by-products from fruit and vegetable processing, grain processing, seafood processing, by-products from the dairy industry, and by-products from edible oil processing, etc. The high protein, oil, and polysaccharide content of oilseed cakes make these readily available, inexpensive, and biodegradable resources a viable base for the environmentally friendly synthesis of biopolymer packaging materials (Popović et al., 2020). These polymers have the potential to successfully replace some of the currently utilized non-biodegradable materials in the food packaging industry (Mirpoor et al., 2022). Among these, wild flax (Camelina sativa), with its abundance and versatility, has emerged as a promising candidate for the development of biodegradable films – *C. sativa* oilcake (CSoC) based biopolymer films (Šuput et al., 2024). The main byproduct of camelina seeds, oilseed cake (pomace) is currently utilized in many different applications: animal feed, functional food, diet supplements, biofuel, and chemical derivatives. Different *C. sativa* fractions have been used to create novel materials or enhance the qualities of already existing materials (*Gursoy et al., 2018*). The continuation of the research of wild flax as a potential packaging material would be its translation into active packaging.

Biopolymer films have an excellent ability to transport bioactive substances, including essential oils, nanoparticles, nutraceuticals, and antimicrobial and antioxidant agents (*Hassan et al.*, 2018). These types of packaging are often referred to as "active packaging materials" and they help enhance food shelf life by preventing the growth of surface microorganisms, reducing oxidation reactions, and maintaining food safety (*Yousefi et al.*, 2018). The integration of essential oils into such biopolymer matrices further adds a dimension of functionality, bringing potential antimicrobial, antioxidant, and aromaenhancing properties (Tian et al., 2023). Essential oils positively impact the characteristics of biopolymer films including antimicrobial properties (Varghese et al., 2020), antioxidant activity (Sivakanthan et al., 2020), aroma enhancement, as well as the customization of biopolymer film properties: characteristics such as flexibility, thermal stability, and barrier properties can be tailored to meet specific application requirements (Reis et al., 2023; Carpena et al., 2021). In this work, two essential oils were selected to activate CSoC based biopolymer films - eucalyptus essential oil (EEO) and rosemary essential oil (REO). Eucalyptus essential oil is derived from the leaves of the eucalyptus tree, primarily native to Australia but now cultivated worldwide. Rich in eucalyptol, the oil exhibits potent antimicrobial and anti-inflammatory characteristics, making it a popular choice for respiratory health support. The primary constituents of eucalyptus essential oil typically include 1,8-cineole (63.1%), p-cymene (7.7%), a-pinene (7.3%), and alimonene (6.9%) (Čmiková et al., 2023). Rosemary essential oil is extracted from the aromatic leaves of the Rosmarinus officinalis plant, a fragrant herb native to the Mediterranean region. The primary constituents of rosemary essential oil include 1,8-cineole (26.54%) and a-pinene (20.14%) (Jiang et al., 2011).

As the global demand for sustainable packaging materials increases, understanding the intricate interplay between the biopolymer matrix and essential oils becomes crucial for the development of films with improved performance and multiple applications (Tomić et al., 2023; Šuput et al., 2019). This research contributes to the growing knowledge of biopolymer science and opens avenues for the development of environmentally friendly packaging solutions in various industries.

In our previous work (*Šuput et al., 2024*), optimal CSoC based biopolymer film synthesis process parameters were defined.

In our previous work (*Šuput et al., 2024*), optimal process parameters for the synthesis of CSoC-based biopolymer films were defined. This scientific work aims to comprehensively characterize CSoC-based biopolymer films activated with essential oils and investigate their physicochemical, mechanical and barrier properties. The study investigates the effects of incorporating essential oils into these films and highlights the synergistic effects resulting from the combination of wild flax biopolymers and natural aromatic compounds.

MATERIAL AND METHOD

Material

Camelina sativa L. seeds were supplied by the Institute of Field and Vegetable Crops (Novi Sad, Serbia). Camelina oil and cake were obtained by seeds cold-pressing. The obtained cake was crushed and sieved by use of a universal laboratory sifter (Bühler AG, Uzwil, Switzerland) equipped with a stack of sieves to obtain fractions smaller than 180 μ m. The basic composition of the obtained fraction was: 39.4% protein, 19.4% oil, and 5.9% cellulose. Eucalyptus essential oil (*Aetheroleum eucalypti*) and rosemary essential oil (*Aetheroleum rosmarini*) were provided by Kirka Pharma (Belgrade, Serbia). All other reagents used in this study were of analytical grade.

Biopolymer film preparation

Biopolymer films based on *Camelina sativa* oilseed cake (CSoC) were prepared by casting method. The filmogenic suspension was prepared by dispersing 5% CSoC in distilled

water with 40% (w/w) plasticizer glycerol. The pH of all samples was further adjusted to pH 10 by adding NaOH solution and determined by pH meter (Metrohm AG, Switzerland). Further, the resulting suspension was incubated at 100 °C for 20 min in a water bath. After heat treatment, the suspension was passed through a nylon filter to remove the cake's coarse undissolved particles. 50 g of film-forming suspension was poured onto Petri plates, covered with Teflon, and allowed to dry for five days at room temperature (23 ± 2 °C, $50\pm5\%$ RH).

Biopolymer film characterization

Film thickness

Film thickness was conducted with a 1 μm sensitivity micrometer DIGICO (TESSA, Switzerland). Eight replicates were carried out on each sample and the result was expressed as mean \pm SD.

Mechanical properties

Tensile strength (TS) and elongation at break (EB) were measured using the Instron Universal Testing Instrument 4301 (Instron Engineering Corp., Canton, MA, USA) following the standard method EN ISO 527-3:2018. The initial distance between the instrument clamps was 50 mm, while the test speed was 50 mm/min. Eight replicates were carried out on each sample and the result was expressed as mean± SD.

Water vapor permeability (WVP)

The water vapor permeability of the film samples was determined according to the standard method ISO 2528:2017. The rate of water vapor transfer through the sample was determined by calculating the difference in the mass of the silica gel before and after treatment (indicating absorbed moisture), dividing it by the surface area of the film sample, and expressing the result in grams per square meter per hour (g/m²h). Three tests were performed for each sample, and the result was expressed as mean \pm SD.

Moisture content

Film samples were cut in squares (surface $1x1cm^2$) and weighed at analytical balance (m_1) . Thereafter, film samples were dried in an air-circulating oven at 105 ± 2 °C (Instrumentaria, Zagreb, Croatia) to a constant weight (m_2) . The moisture content (MC) is the percentage of weight reduction after film drying, expressed on the total weight of the film. It was determined as:

MC (%) = $(m_1 - m_2)/m_1 * 100$

The result was expressed as the mean value of three independent measurements \pm SD, for each sample.

Film solubility

Immediately after determining the moisture content, the same film samples $(1 \times 1 \text{ cm}^2)$ that were dried to constant mass, were used to determine the film solubility. Each film sample was weighed (m_1) and immersed in 30 mL of deionized water and allowed to stand for 24h at room conditions. After 24 h, the water was decanted and the films were dried again in an aircirculating oven (Instrumentaria, Zagreb, Croatia) at (105 ± 2) °C to a constant mass (m_2) . The total solubility of the films (%) was calculated:

S (%) = $(m_1 - m_2)/m_1 * 100$

The result was expressed as the mean value of three independent measurements \pm SD, for each sample.

Antioxidative activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

In a bottle containing 2 mL of a freshly prepared DPPH• solution (0.0185 g DPPH• in 50mL ethanol) diluted in ethanol (1:5), a film sample $(1x1 \text{ cm}^2)$ was placed and the mixture was stirred for 24 h in a dark chamber at room temperature. In each

sample, the residual DPPH• concentrations were determined after the removal of the solid film and by measuring the absorbance at 520 nm using a T80/T80+UV-VIS spectrophotometer (PG Instruments LTD, UK). The control sample (blank) is the sample in which there was no film. The antioxidant activity of the films was expressed as a percentage and calculated according to the following formula:

AO (%) = $((DPPH_{\bullet_0} - DPPH_{\bullet_s}))/(DPPH_{\bullet_0}) * 100$

where DPPH•s is the concentration of DPPH• in the tested film sample and DPPH•₀ is the concentration of DPPH• in the corresponding blank. The result was expressed as the mean value of three independent measurements \pm SD, for each sample.

Statistical analysis

Statistical analysis was carried out by the software package StatSoft Statistica ver. 10.0. All data were presented as mean values with their standard deviation indicated (mean \pm SD).

Variance analysis (ANOVA) was performed, with a confidence interval of 95 % (p < 0.05). Means were compared by the Tukey test.

RESULTS AND DISCUSSION

Film thickness

The thickness of CSoC based biopolymer films enriched with essential oils ranged from 0.33 to 0.36 mm, while the control film had a thickness of 0.32 mm (Table 1). It was observed that thickness changed significantly with the addition of essential oils. One explanation would be that the inclusion of EOs in the film matrix enhanced molecular interactions like hydrogen and covalent bonds, which resulted in a denser film and a more compact film structure. Akhter et al. (2019) who examined the addition of lactic acid, mint EO and rosemary EO to chitosan-pectin edible films reported similar results.

Table 1. Mechanical and physico-chemical properties of Camelina sativa based films with the addition of rosemary and eucalyptus essential oils

Sample	MC (%)	S (%)	d (mm)	TS (MPa)	EB (%)
K	$25.93{\pm}1.42^{b}$	39.56±1.39 ^c	0.32 ± 0.004^{a}	$2.28{\pm}0.08^d$	8.83 ± 0.14^{a}
E 0.5	$22.04{\pm}0.29^{a}$	33.14±2.05 ^b	0.33±0.003 ^{ab}	2.15 ± 0.14^{cd}	10.32±1.26 ^{ab}
E 1	23.42 ± 0.87^{a}	27.42 ± 1.32^{a}	0.35±0.002 ^c	$2.05 \pm 0.10^{\circ}$	11.31±1.17 ^{bc}
E 2	22.16±0.48 ^a	29.08 ± 1.76^{ab}	0.36±0.002 ^c	1.63 ± 0.07^{b}	12.66±1.47 ^{bc}
R 0.5	23.08±0.43 ^a	26.21±0.71 ^a	0.34 ± 0.008^{b}	$1.40{\pm}0.04^{a}$	11.97±1.66 ^{bc}
R 1	22.15 ± 0.97^{a}	29.99 ± 2.22^{ab}	0.36±0.003 ^c	1.69±0.11 ^b	11.58±0.75 ^{bc}
R 2	23.77±0.33 ^{ab}	28.34±1.13 ^a	0.35±0.003°	1.29 ± 0.09^{a}	14.29±0.23 ^d

^{a-d} Means in the same column with different superscripts are statistically different ($p \le 0.05$)

 $MC \ (\%) \ - \ moisture \ content, \ S \ (\%) \ - \ solubility, \ d \ (\mu m) \ - \ thickness, \ TS \ (MPa) \ - \ tensile \ strength, \ EB \ (\%) \ - \ elongation \ at \ break \ break \ - \ - \ break \ - \ break \ - \ break \ - \ break \ - \ break$

Mechanical properties

The effect of EO addition on the mechanical properties of CSoC based biopolymer films was presented in Table 1 by analyzing tensile strength (TS) and elongation at break (EB). The highest level of tensile strength was noticed in the control film with no EO, followed by the EO-added films. The addition of EOs caused TS to decrease from 2.28 MPa to 1.63 MPa for eucalyptus essential oil and from 2.28 MPa to 1.29 MPa for film samples with added rosemary essential oil. Also, the higher the content of oil, the lower the obtained values of tensile strength. Similar results were obtained by Venkatachalam et al. (2023) and Galus and Kadzińska (2016). The introduction of lipids into the film matrix results in a discontinuous, heterogeneous film structure. Depending on the characteristics of the additional lipid, this influences the films' tensile properties. The principal explanation for the strength reduction might be attributed to the film network's partial substitution of weaker polymer-oil connections for stronger polymer-polymer interactions. Theoretically, this TS decrease may be accompanied by either an increase or a decrease of %EB (Atarés and Chiralt, 2016).

In this case, the elongation at the break of the tested film exhibited a contrary effect against the tensile strength. The least elongation at break was noticed in the control film (8.83%), indicating lesser flexibility, followed by the EO-added samples (14.29% for CSoC based biopolymer film with the addition of 2% eucalyptus essential oil). This means that the incorporation of both EOs resulted in a less rigid film matrix, with enhanced film stretchability. Also, the higher the content of oil, the higher the obtained values of elongation at break. The inclusion of essential oils in protein-based or polysaccharide-based films can interfere with polymer chain-to-chain interactions and improve cohesiveness producing flexible domains within the film that propose better extensibility (*Lorevice et al.*, 2016).

Water vapor permeability

Biopolymer films based on proteins, polysaccharides, or even composites, such as CSoC films, are known to have poor moisture barrier properties due to their hydrophilic nature. One strategy to reduce water sorption and water transfer through films is to incorporate a hydrophobic phase consisting of essential oils into the film matrix. Water vapour transfer in films depends on the ratio of hydrophilicity to the hydrophobicity of the film components (Atarés and Chiralt, 2016). Lipid incorporation is expected to increase the hydrophobicity of these films and reduce the water vapour permeability (Rocca-Smith et al., 2016). Our results showed that the incorporation of natural EOs had a significantly decreasing effect on the WVP of CSoC based biopolymer films. The control film had a WVP of 5.43 g/m^2h , while the lowest WVP values were recorded for the film sample with 2% added eucalyptus essential oil (3.14 g/m²h), Figure 1. At all oil concentrations, the water vapour permeability had lower values, and a dose-dependent tendency with increasing oil content was also observed. The same conclusion was observed in studies of Akhter et al. (2019), Galus and Kadzińska (2016), and Mahdavi et al. (2018). The undelaying mechanism relies on the fact that the incorporation of lipids into a film results in a large number of spherical particles uniformly dispersed throughout the matrix, which increases the distance a permeating molecule must travel to pass through the film (Castro-Rosas et al., 2016).



Fig. 1. Water vapour permeability (g/(m2·h) of Camelina Sativa based films with the addition of rosemary and eucalyptus essential oils

Moisture content and film solubility

The control film (K) showed the highest moisture content (25.93%) and water solubility (39.56%) (Table 1). A significant decrease in moisture content and water solubility was observed for films upon the addition of eucalyptus and rosemary essential oils to CSoC based biopolymer films. The MCs in the tested films with added essential oils didn't significantly differ. The lower solubility of CSoC based biopolymer films might be the result of the hydrophobic nature of lipid and lipid-polysaccharide interactions mainly via hydrogen bonding. Due to this mechanism, hydroxyl groups are less available to interact with water molecules, which results in the production of more water-resistant films. The obtained results are in agreement with other author's findings (*Akhter et al., 2019; Venkatachalam et al., 2023*).

Antioxidative activity

Figure 2 presents the antioxidant activity of all the films studied, which was determined by the disappearance of DPPH radical absorbance at 517 nm. The results indicate that all the examined film samples had free-radical scavenging capacity. Interestingly, the control film exhibited 60.10% antioxidant activity. This high antioxidant value could be attributed to the presence of phenolic compounds that cannot be removed because of their strong interaction with proteins. Nearly all of the phenolic chemicals found are still present in the seed cake after the oil has been extracted from the seeds (Terpinc et al., 2012). The synergism between polyphenols and water-soluble amino acids and their derivatives speaks for a high antioxidant activity, suggesting a high level of antioxidant activity (Jin et al., 2016). It has been reported that six flavonoids (catechic acid, epicatechin, quercitrin, isoquercetin, rutin and isorhamnetin), three hydroxybenzoic acids (proto-catechuic acid, phydroxybenzoic acid and salicylic acid) and one hydroxycinnamic acid (sinapic acid) have been identified in camelina oil cake (Li et al., 2023). The addition of EOs ranging from 0.5% to 2% significantly enhanced the antioxidant activity. The antioxidant activity for CSoC based biopolymer film samples with rosemary essential oil was in the range of 63.00-69.82% and for the samples with eucalyptus essential oil was 56.87-78.54%. The highest level of antioxidant activity was related to the sample with 2% eucalyptus oil added. The obtained results indicate that all synthesized films could be used as active films with significant antioxidant activity.



Fig. 2. Antioxidative activity (%) of Camelina Sativa based films with the addition of rosemary and eucalyptus essential oils

CONCLUSION

In this paper, the influence of two essential oils (eucalyptus and rosemary) on the properties of CSoC based biopolymer films was examined. It was confirmed that the addition of essential oils affects the mechanical properties so that samples with a higher concentration of added oils showed lower tensile strength and higher elongation at break values. Also, the addition of both essential oils contributed to the improvement of the barrier properties of the films against water vapor because the values were significantly reduced. Control film inhibited high antioxidant activity, which was improved by essential oils addition in a dose-dependent manner. Both in the case of barrier properties and in the case of tested antioxidant properties, eucalyptus essential oil proved to be more effective than rosemary oil.

ACKNOWLEDGMENT: This paper is the result of a project "Proizvodnja i primena bio-ambalaže dobijene iz agroindustrijskog otpada (Production and application of biopackaging obtained from agro-industrial waste)" financed by the Provincial Secretariat for Higher Education and Scientific Research, Autonomous Province of Vojvodina, project number142-451-3059/2023-01/02 and Program number 451-03-66/2024-03/200134 financed by the Ministry of Science, Technological Development and Innovation.

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Received: 19. 02. 2024.

Accepted: 04. 04. 2024.