# Incorporation of essential oils into pumpkin oil cake-based materials in order to improve their properties and reduce water sensitivity

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*Abstract*

Biopolymer-based materials present good alternatives for synthetic materials. However, their high water sensitivity may limit their usage for food products packaging. Addition of hydrophobic components into the material formulation could improve this property. In this work 3, 4 and 5 % (v/v) of *Satureja montana* or *Ocimum basilicum* essential oil was incorporated into biopolymer films based on pumpkin oil cake. The obtained materials were analyzed regarding mechanical, physicochemical, barrier and structural properties. Incorporation of the essential oils increased the thickness of the pumpkin oil cake film. Significant reductions in moisture sensitivity, related to physicochemical properties and water vapor transmission rate (almost for 30 %), were observed ($p < 0.05$). Improvement of light barrier properties was also observed so that the visible light transmission was decreased for around 50 % while the UV light transmission was lower than 1 %. The obtained FTIR spectra confirmed the presence of added essential oils in pumpkin oil cake films, as well as their influence on the reduction in the film surface hydrophilicity. However, mechanical properties, tensile strength and elongation at break, decreased significantly ($p < 0.05$). These results suggest that incorporation of *Satureja montana* or *Ocimum basilicum* essential oil improved barrier properties of pumpkin oil cake-based films and reduced the film affinity toward water.

*Keywords:* biopolymer materials; lipids; mechanical properties; physicochemical properties; barrier properties; FTIR.

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## 1. INTRODUCTION

Biopolymer-based materials are promising alternatives for synthetic plastic materials [1]. They are obtained from natural sources such as proteins, polysaccharides and lipids so that they could be even edible, while after disposal in environment they are decomposed due to the influence of external factors. It was proven that protein and polysaccharide based materials have good mechanical and organoleptic properties, present effective barriers to aroma compounds, light and gases, and can retard oil and fat migration, or serve as carriers for food additives. However, due to the hydrophilic nature these materials exhibit high water sensitivity and show low barrier properties toward water. In contrast, lipid-based films show low water sensitivity and good moisture blockage, but are brittle with poor mechanical and undesirable sensory properties [2–6]. Use of composite packaging materials could be a promising solution for packaging and protection of different food products. Proteins or polysaccharides could provide a good mechanical resistance and addition of lipids could decrease the water sensitivity of these materials.

Pumpkin oil cake (PuOC) is a by-product obtained after oil extraction from pumpkin seed (*Cucurbita pepo* L.) by cold pressing and it represents an important oil crop in Serbia. It is a natural complex blend of mainly proteins (around 63 %), polysaccharides and lipids [7]. Since it is obtained from agro-industrial waste/by-product, use of PuOC for biopolymer-

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based film development, could contribute to the reduction of environmental waste. Even though PuOC contains around 8.4 % of oils, the water sensitivity of PuOC-based films is still very high [7].

One way to improve water sensitivity and barrier properties of biopolymer-based films is to include hydrophobic components into their formulations [4]. Many researchers have used different compounds to improve water sensitivity and water vapor permeability of biopolymer films such as waxes [8,9], phenolic compounds [10,11], essential oils [12-14], extracts [15], *etc.* Essential oils are natural blends, which were proved to be good sources of bioactive compounds with pronounced antimicrobial and antioxidant properties mostly due to the presence of phenolic compounds. Additionally, most of these oils are classified as Generally Recognized as Safe [16]. Aromatic plants *Satureja montana* L. and *Ocimum basilicum* L. belong to the *Lamiaceae* family. They are widely spread in the Balkan region and can be found in Serbia. The major and the most abundant phenolic compounds in the *Satureja montana* essential oil are usually carvacrol and thymol responsible for its biological properties [17,18]. The major compounds in the *Ocimum basilicum* essential oil are linalool, methyl chavicol and eugenol, known to exhibit various biological activities such as antimicrobial, antioxidant, anti-inflammatory and anticancer [19]. All these components are highly hydrophobic phenolic monoterpenes, and addition of the mentioned essential oils could improve water sensitivity and other properties of biopolymer-based materials.

The aim of this research was to develop biopolymer composite films based on the agro-industrial by-product pumpkin oil cake and lipophilic compounds *Satureja montana* and *Ocimum basilicum* essential oils. In addition, influence of the oil addition on mechanical, physicochemical, barrier and structural properties of the PuOC-based films was evaluated. The experiment was performed with the aim to evaluate potentials of the essential oil addition for improvement of the film properties and sensitivity to water.

## 2. MATERIALS AND METHODS

### 2. 1. Materials

The pumpkin oil cake (*Cucurbita pepo* L.) was provided from *Linum* d.o.o., Čonoplja, Serbia and *Satureja montana* and *Ocimum basilicum* essential oils were supplied from *Institute of Field and Vegetable Crops*, Novi Sad, Serbia. All other reagents used in this study were of analytical grade.

### 2. 2. Preparations of films

Films were prepared by a casting method. First 10 % (w/w) film-forming suspension of the pumpkin oil cake (PuOC) with glycerol (30 % (w/w), per weight of PuOC) used as a plasticizer, and guar-xanthan (0.2 % (w/w), per weight of polysaccharides in PuOC) used as a surfactant, in deionized water, was prepared. After adjusting the pH value to 10 using 0.2 M NaOH, and incubating at 60 °C for 20 min, the film-forming suspension was filtrated and *Satureja montana* or *Ocimum basilicum* essential oil was added to result in predetermined concentration: 3, 4 and 5 % (v/v). The suspension was homogenized by an Ultra Turrax homogenizer (SilentCrusher M, Heidolph, Germany), for 2 min at 20,000 rpm 2 times, and casted onto Teflon-coated Petri dishes. A film without addition of essential oils was used as a control. After drying, the obtained films were peeled off, conditioned at room conditions (23±2 °C, 50±5 % RH) and analyzed. Films were labeled as: 3%Sm, 4%Sm and 5%Sm for PuOC-based films with 3, 4 and 5 % (v/v) of *Satureja montana* essential oil, respectively; and 3%Ob, 4%Ob and 5%Ob for PuOC-based films with 3, 4 and 5 % (v/v) of *Ocimum basilicum* essential oil, respectively. Ten films for each sample were prepared, which was sufficient for all analyzes.

### 2. 3. Characterizations of the obtained films

#### 2. 3. 1. Visual appearance

The obtained active films were visually observed before analyzing the mechanical, physicochemical, barrier and structural properties. Color, odor and visual appearance of the film surface were examined, and the effect of the essential oil type as well as the added concentration (3-5 %) on visual characteristics of PuOC based films was determined.

### 2. 3. 2. Thickness

The film thickness was measured by a micrometer (Digico 1, Tesa, Swiss Made, Renens, Switzerland), with a sensitivity of 0.001 mm, at 15 positions on five films from each sample and the results were expressed as the mean value.

### 2. 3. 3. Mechanical properties

Mechanical properties, *i.e*. tensile strength (TS) and elongation at break (EAB) of samples, were measured by an Instron Universal Testing Instrument Model 4301 (Instron Engineering Corp., Canton, MA) according to the ASTM standard method [20]. The samples were cut as the rectangular film strips of 80 mm in length and 15 mm in width. The initial grip separation was set at 50 mm, and the crosshead speed set at 50 mm min$^{-1}$. Measurements of the mechanical properties for each film type were repeated at least five times and mean values were calculated.

### 2. 3. 4. Physicochemical properties - swelling degree

Physicochemical properties (swelling degree, moisture content and the total soluble matter) of the obtained films were determined according to Hromiš *et al.* [9].

For determination of the swelling degree, samples, 1×2 cm in size, were weighed ($m_1$) and then immersed in a 25 cm$^3$ of distilled water for 2 min under shaking. Subsequently, water was decanted, and excess water was removed from the sample by a filter paper, followed by reweighing the mass of the sample ($m_2$), and the swelling degree was calculated according to the equation:

$$\text{Swelling degree} = \frac{m_2 - m_1}{m_1} 100 \tag{1}$$

For each sample, the test was repeated at least three times, and the swelling degree of the samples was expressed as mean value.

### 2. 3. 5. Physicochemical properties – moisture content

To determine the moisture content (MC), the samples 2×2 cm were weighed ($m_1'$) and dried at 105±2 °C to constant mass designated as $m_3$. The MC values were expressed as mean value of three measurements for each sample, and calculated according to the equation:

$$\text{Moisture content } = \frac{m_1' - m_3}{m_1'} 100 \tag{2}$$

### 2. 3. 6. Physicochemical properties – total soluble matter

To determine the total soluble matter (TSM) contents, samples 2×2 cm, were dried in an oven at 105±2 °C to constant mass ($m_1''$). After measurement, each film was left in 50 cm$^3$ of distilled water for 24 h at room temperature, under occasional shaking. After this period, water was decanted and the films were dried again in the oven at 105±2 °C to constant mass ($m_4$). TSM (%) was calculated according to the following equation:

$$\text{Total soluble mater } = \frac{m_1'' - m_4}{m_1''} 100 \tag{3}$$

The test was repeated in three independent replicates and the results are presented as mean values.

### 2. 3. 7. Barrier properties– Water vapor transmission rate

The water vapor transmission rate (WVTR) was determined by the gravimetric method according to the ISO standard [21]. The used test conditions were: temperature 25±1 °C and the relative humidity of 90±2 %. Three replicates for each sample were determined and the results are expressed as mean values.

2. 3. 8. Barrier properties– Light permeability

Light permeability of the obtained films was determined by using a T80 + UV/VIS spectrophotometer (PG instruments LTD, United Kingdom), in the wavelength range from 200 - 800 nm, according to Hosseini *et al.* [22].

2. 3. 9. Structural properties – FTIR analysis

Fourier-transform infrared (FTIR) spectra of the samples were recorded at room temperature by using a Nicolet IS10 FT-IR spectrophotometer (Thermo Fisher Scientific, MA, USA) operating in total reflectance attenuation (ATR) mode, according to Hromiš *et al.* [9]. All spectra were recorded in the spectral range 4000-500 cm$^{-1}$, at a resolution of 4 cm$^{-1}$. Omnic 8.1. Software (Thermo Fisher Scientific, MA, USA) was used to collect, manage, and process all the data.

### 2. 4. Statistical analysis

Statistical analysis was carried out using Statistica 13.5.0.17 (TIBCO Software Inc., Palo Alto, CA, USA). All the data were presented as mean values, at three significant numbers, with the standard deviation indicated. The variance analysis (ANOVA) was performed, with a confidence interval of 95 % ($p < 0.05$), and the means obtained were compared by the Tukey test.

## 3. RESULTS AND DISCUSSION

### 3. 1. Visual appearance

Films were greenish in color, which originates from the substrate (PuOC), turbid and flexible (Fig. 1). A yellowish hue has occurred in films with added essential oils, especially in films with the *Satureja montana* essential oil. With the increase in oil concentration, the intensity of yellowish color also increased. The control film had mild odor on PuOC, while films with the oils had pronounced odors of the added essential oils, which increased with increasing the oil concentration, with a minor hint of PuOC. Films with higher oil concentrations, especially those with 5 %, had reduced homogeneity and rough and uneven surfaces, due to insufficient oil dispersion. However, pores or cracks on the film surfaces were not observed in any of the samples.

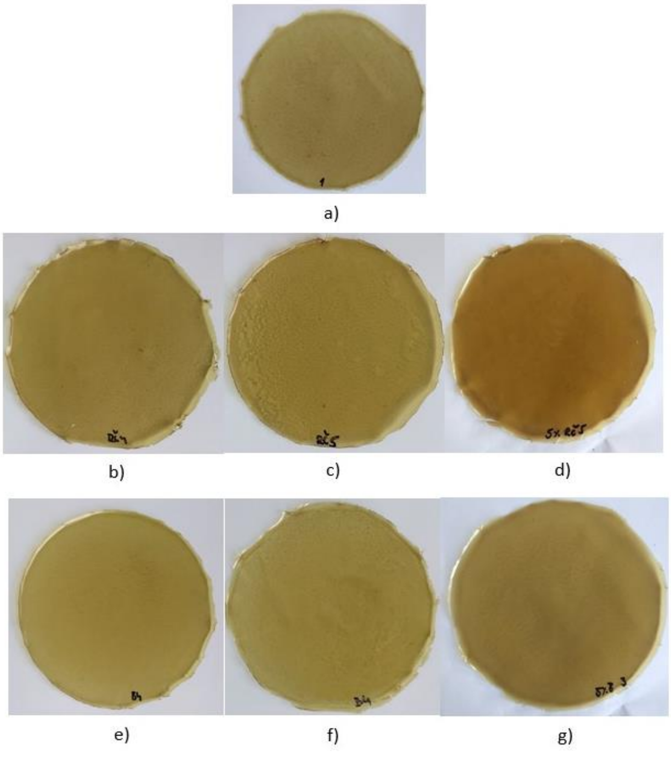
a)
b) c) d)
e) f) g)

*Figure 1. Photographs of biopolymer films based on PuOC: a) control film without essential oils; b), c), d) with 3, 4 and 5 % of Satureja montana essential oil, respectively; and e), f), g) with 3, 4 and 5 % of* Ocimum basilicum *essential oil, respectively*

### 3. 2. Thickness

Thickness of a biopolymer film is an important characteristic that directly affects the other properties, its potential application and product sustainability [23].

The measured thicknesses of the obtained PuOC-based films are presented in Table 1. Thickness of the control film was ~150 µm, and for PuOC films with essential oils was in the range from 160 µm for 4%Sm to ~200 µm for 5%Ob, indicating that the incorporation of those essential oils significantly affected the film thickness ($p < 0.05$), and with increasing the essential oil concentration, the film thickness also increased. Those results are in correlation with similar literature data [24–27], suggesting that the biopolymer film thickness increases by increasing the number of dissolved compounds, attributed to the increase in the solid content.

*Table 1. Thickness, tensile strength (TS) and elongation at break (EAB) of biopolymer films based on PuOC*

| Film | Thickness, µm | TS, MPa | EAB, % |
|---|---|---|---|
| control | 148 ± 2$^{a}$ | 0.638 ± 0.008$^{a}$ | 101 ± 2$^{a}$ |
| 3%Sm | 164 ± 1$^{b}$ | 0.576 ± 0.011$^{b}$ | 47.7 ± 3$^{d}$ |
| 4%Sm | 160 ± 1$^{b}$ | 0.556 ± 0.027$^{b}$ | 53.9 ± 6$^{bcd}$ |
| 5%Sm | 172 ± 1$^{c}$ | 0.452 ± 0.015$^{c}$ | 59.3 ± 4$^{b}$ |
| 3%Ob | 163 ± 3$^{b}$ | 0.548 ± 0.028$^{b}$ | 53.1 ± 3$^{bcd}$ |
| 4%Ob | 170 ± 2$^{c}$ | 0.482 ± 0.005$^{c}$ | 58.1 ± 1$^{bc}$ |
| 5%Ob | 196 ± 4$^{d}$ | 0.381 ± 0.018$^{d}$ | 50.0 ± 2$^{bcd}$ |

Data are mean values ± standard deviation. Different superscripts indicate significant differences among samples in columns ($p < 0.05$). a - all samples in column containing letter a are not significantly different ($p > 0.05$); b - all samples in column containing letter b are not significantly different ($p > 0.05$); c - all samples in column containing letter c are not significantly different ($p > 0.05$); d - all samples in column containing letter d are not significantly different ($p > 0.05$)

### 3. 3. Mechanical properties

Mechanical properties of packaging materials are important with respect to possible applications for food products indicating the material resistance during the use. Most commonly reported mechanical properties of packaging materials are tensile strength (TS), that represents the material strength and the maximum tensile stress that the material can resist, and elongation at break (EAB) that represents the maximum change in length of the sample before break [16,28,29]. When it comes to mechanical properties of biopolymer packaging materials, they can be affected by many factors, primarily by the film composition, that is the choice of the biopolymer used as a substrate, surfactants, plasticizers and additives, and their relative proportions and interactions, as well as the preparation conditions such as temperature, pH, homogenization, drying and storage conditions [28,30].

TS and EAB of the obtained biopolymer materials are presented in Table 1. TS of the control film, without added essential oils, was ~0.64 MPa, and EAB 101 %. The biopolymer film based on PuOC thus has shown a lower TS value as compared to those reported for other biopolymer protein films [5,24,30–36], but the EAB value was comparable or higher as compared to the values reported for other protein-based films [5,24,30–36].

Incorporation of essential oils into biopolymer films based on PuOC significantly decreased its mechanical properties ($p < 0.05$), and with the increase in the essential oil concentration, the reduction of TS and EAB was more pronounced. Higher TS reduction was observed with addition of the *Ocimum basilicum* essential oil as compared to films with the *Satureja montana* essential oil, especially at the concentration of 5 %, at which TS decreased from 0.638 MPa (control) to 0.381 MPa (5%Ob). Decrease of TS of biopolymer films based on proteins caused by addition of essential oils, was already reported in the literature [22,24,30,31,34,36].

Incorporation of hydrophobic substances into film-forming solutions can lead to formation of new polymer-oil bonds in the film network, that are usually much weaker than polymer-polymer interactions [37]. Such polymer-oil bonds could result in increased pore sizes, creation of possible rupture points, as well as heterogeneity and discontinuity in the film structure, leading to a decrease in mechanical resistance [38].

In this work, according to the results presented in Table 1, addition of *Satureja montana* and *Ocimum basilicum* essential oils, resulted in the EAB reduction of the obtained films was for almost 50 %, without significant differences at different essential oil concentrations ($p > 0.05$). The same observation was reported for biopolymer protein films based on gelatin with oregano and lavender essential oils [34], hake protein with thyme [30] and tarragon essential oils [31], and casein films with *Matricaria recutita* essential oil [24].

### 3. 4. Physicochemical properties

Physicochemical properties, the moisture content (MC), total soluble matter (TSM) and swelling degree of the obtained PuOC-based films are shown in Table 2. These material properties, together with WVTR, are related to the water and moisture sensitivity of the film, that is one of the major challenges when it comes to application of biopolymer-based films.

*Table 2. Moisture content (MC), total soluble matter (TSM) and swelling degree of biopolymer films based on PuOC*

| Film | MC, % | TSM, % | Swelling degree, % |
|---|---|---|---|
| control | $19.8 \pm 0.6^{a}$ | $36.0 \pm 0.8^{ab}$ | $148 \pm 6^{a}$ |
| 3%Sm | $17.8 \pm 0.9^{ab}$ | $37.8 \pm 0.6^{b}$ | $109 \pm 3^{b}$ |
| 4%Sm | $17.2 \pm 1.4^{b}$ | $37.7 \pm 0.6^{b}$ | $108 \pm 10^{bc}$ |
| 5%Sm | $19.2 \pm 0.8^{ab}$ | $35.9 \pm 2.3^{ab}$ | $106 \pm 3^{bc}$ |
| 3%Ob | $18.9 \pm 0.3^{ab}$ | $33.8 \pm 0.7^{a}$ | $112 \pm 11^{b}$ |
| 4%Ob | $17.1 \pm 0.5^{b}$ | $34.3 \pm 1.1^{ab}$ | $105 \pm 5^{bc}$ |
| 5%Ob | $14.4 \pm 1.1^{c}$ | $36.6 \pm 1.7^{ab}$ | $93.9 \pm 3^{c}$ |

Data are mean values ± standard deviation. Different superscripts indicate significant differences among samples in columns ($p < 0.05$). a - all samples in column containing letter a are not significantly different ($p > 0.05$); b - all samples in column containing letter b are not significantly different ($p > 0.05$); c - all samples in column containing letter c are not significantly different ($p > 0.05$)

According to the obtained results, the highest impact of the essential oil addition, was observed for swelling properties, where the swelling degree significantly decreased with essential oil addition as compared to the control film ($p < 0.05$). Significant reductions in the percentage of swelling were achieved by adding 5 % of either *Satureja montana* or *Ocimum basilicum* essential oils into PuOC-based film (for 28 and 37 %, respectively as compared to the control film, Table 2). Different concentrations of the *Satureja montana* essential oil did not show significant influence ($p > 0.05$) on the swelling degree of PuOC-based films. Incorporation of hydrophobic essential oils into biopolymer-based films could increase interactions between polymers in the film and oil components, and increase surface hydrophobicity of the films, thereby reducing the affinity of the films toward water [17,39].

With the addition of *Ocimum basilicum* essential oil at concentrations in the range 3 to 5 %, significant reductions in the MC were achieved down to 14.4 % (5%Ob) as compared to that of the control film (19.8 %). Similar results were reported for incorporation *Matricaria recutita* essential oil into a casein-based film [24] and olive oil into a gelatin-based film [40]. The MC decrease in biopolymer-based films with addition of essential oils is a consequence of the oil hydrophobic nature, causing lower water absorption by the film matrix. However, incorporation of the *Satureja montana* essential oil into PuOC-based films did not induce significant differences in MC ($p > 0.05$).

TSM of packaging materials is related to water resistance and is very important in food protection especially for products exhibiting high water activity [22]. TSM of the control film was 36.0 %, and according to the literature data, PuOC-based films exhibit low water-solubility compared to other protein-based films with higher TSM values, such as 98 % for gelatin [36], 86 % for casein [24], 66 % for hake proteins [31], and 93.2 % reported for sunflower proteins [35]. According to the obtained results presented in Table 2, incorporation of different concentrations (from 3 to 5 %) of *Satureja montana* and *Ocimum basilicum* essential oils into PuOC-based film, did not have significant influence on TSM ($p > 0.05$).

Higher reduction in the film water sensitivity observed as swelling and MC properties, with the addition of *Ocimum basilicum* essential oil as compared to those observed upon addition of *Satureja montana* essential oil, could be because of the higher hydrophobicity in the former case, that was also confirmed by the FTIR analysis (Subsections 3.6).

## 3. 5. Barrier properties

### 3. 5. 1. Water vapor transmission rate

High water vapor permeability (WVP) or water vapor transmission rate (WVTR) can be a major drawback of packaging materials aimed for food products sensitive to moisture content. Biopolymer packaging materials are usually hydrophilic with polar groups in their molecular structures, which affect their WVP and WVTR, being much higher as compared to those of synthetic materials [41]. WVTR values of the obtained PuOC-based films are presented in Figure 2.

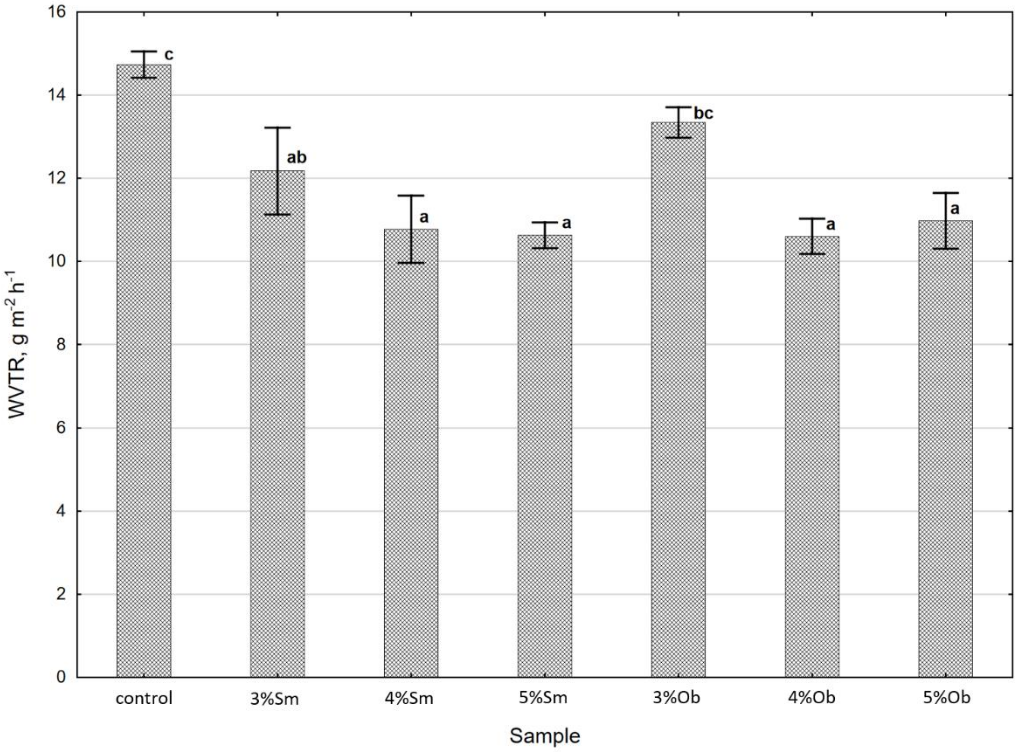

*Figure 2. Water vapor transmission rate (WVTR) of biopolymer films based on PuOC. Data are mean values ± standard deviation. Different lower case letters indicate significant differences among the samples (*p* < 0.05). a - all samples containing letter a are not significantly different (*p* > 0.05); b - all samples containing letter b are not significantly different (*p* > 0.05); c - all samples containing letter c are not significantly different (*p* > 0.05).*

Incorporation of essential oils significantly reduced WVTR of PuOC materials ($p < 0.05$, Fig. 2). The oil concentration of 3 % induced a WVTR decrease from 14.7 g m$^{-2}$ h$^{-1}$ for the control to 12.2 g m$^{-2}$ h$^{-1}$ for 3%Sm, and to 13.3 g m$^{-2}$ h$^{-1}$ for 3%Ob. At higher oil concentrations (4 and 5 %) the WVTR reduction was even more pronounced, regardless of the type and concentration of the added essential oil ($p > 0.05$). At the highest oil concentration investigated of 5 %, the WVTR reduction was around 28 % (to 10.6 g m$^{-2}$ h$^{-1}$) and 26 % (10.9 g m$^{-2}$ h$^{-1}$) for *Satureja montana* and *Ocimum basilicum* essential oils, respectively. Similarly, WVP reductions were reported for different biopolymer based films upon addition of various essential oils such as gelatin-based films with lemongrass oil [36], oregano and lavender essential oils [34], bergamot, kaffir lime, lemon, lime essential oils [32] and ginger, turmeric and plai essential oils [42], as well as casein-based films with *Matricaria recutita* essential oil [24], hake proteins – based films and thyme oil [30]. Water vapor transfer generally occurs through the hydrophilic portion of the film and depends on the hydrophilic/hydrophobic ratio of the film components [17]. Essential oils are composed mainly of monoterpenes that represent highly hydrophobic substances [43]. Thus the addition of essential oils into a biopolymer film could increase the film hydrophobicity, thereby reducing the water vapor migration through the film [42].

### 3. 5. 2. Light permeability

Light permeability is another very important barrier property of packaging materials, beside WVTR, in order to protect foods against oxidation, nutrient losses and unpleasant flavors [39,42]. Transmission of the obtained PuOC materials in the wavelength range of UV and visible light from 200 to 800 nm is presented in Figure 3.

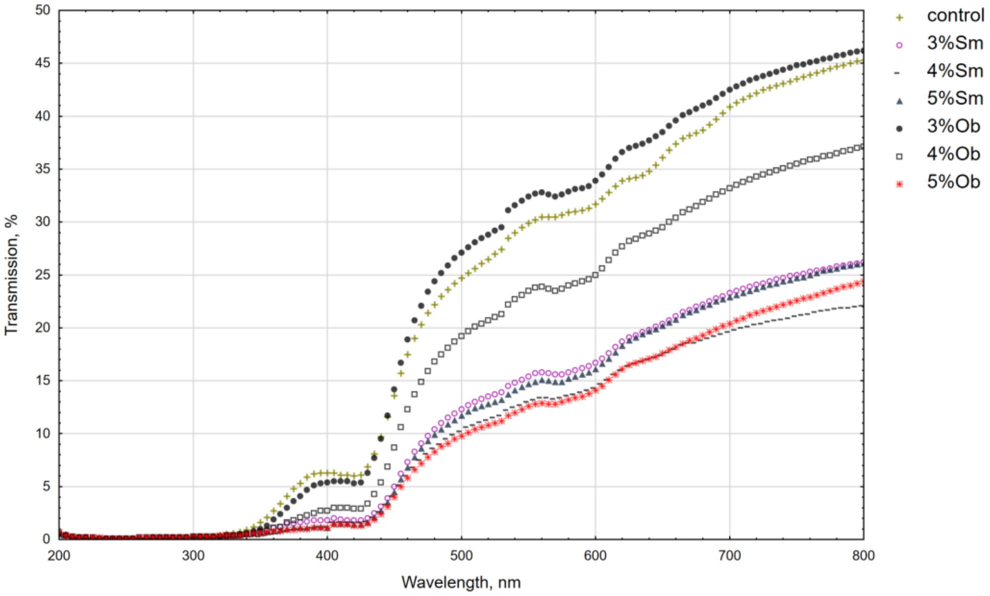

*Figure 3. Light permeability of biopolymer films based on PuOC at wavelengths ranging from 200 to 800 nm*

All biopolymer materials had transmission lower than 1 % in the wavelength range from 280 to 200 nm, typical for the area of UV radiation. This part of the light spectrum generally initiates various reactions in the packed product, that cause a change in the product quality. In previous studies [22, 41] authors suggested that low transmission of protein-based films is probably due to the high content of aromatic amino acids, which absorb the UV light, such as tyrosine, phenylalanine and tryptophan. The presence of different amino acids, such as phenylalanine, has also been reported in amino acidic composition of PuOC [23]. Nonetheless, the green pigment present in PuOC, could also contribute to the reduction of light transmission.

Transmission of the control film in the visible light wavelength range, from 350 to 800 nm, ranged between 1.6 % (350 nm) and 45.3 % (800 nm). Compared to the literature data, the PuOC-based film shows significantly lower light permeability as compared to other protein films. For example at 350 nm for gelatin-chitosan films light permeability values of ~56 % [45] and 62 % [22] were reported while 75 % was reported for a fish skin gelatin film [42]. At 800 nm, light permeability values of those protein films, varied from around 80 % for gelatin-chitosan films [34,43] to 90 % for the fish skin gelatin film [42].

With incorporation of essential oils into PuOC-based films, light transmission decreased in a concentration dependent manner. With the addition of 4 % *Satureja montana* essential oil transmission at the wavelength of 800 nm decreased for 51 % that is from 45.3 % for the control to 22.1 %, while with the addition of 5 % *Ocimum basilicum* essential oil it decreased for 46 % that is to 24.4 %. Decrease of light permeability with addition of different essential oils was also reported in literature [22,26,32,36,42,46]. Ramziia *et al.* [45] suggested that a decrease in light permeability of biopolymer films upon incorporation of essential oils could be because of the presence of polyphenol components containing a benzene ring that enhances the light absorption.

### 3. 6. Structural properties – FTIR analysis

Fourier-transform infrared spectroscopy (FTIR) is a useful technique for monitoring structural changes on the molecular level of samples, as well as for determining the microstructural characteristics of biodegradable films especially composites, as it defines the existing bonds in the film matrix molecules and formed bonds between different film components [23].

FTIR spectra of PuOC-based films with *Satureja montana and Ocimum basilicum* essential oils are presented in Figures 4 and 5, respectively. In general, all spectra of PuOC-based films showed major bands that are characteristic for protein molecules: peaks obtained in the 3700-3000 cm$^{-1}$ region, correspond to the amide A (around 3500 cm$^{-1}$) and amide B (around 3100 cm$^{-1}$) region, originating from the bonds formed between amino acids.

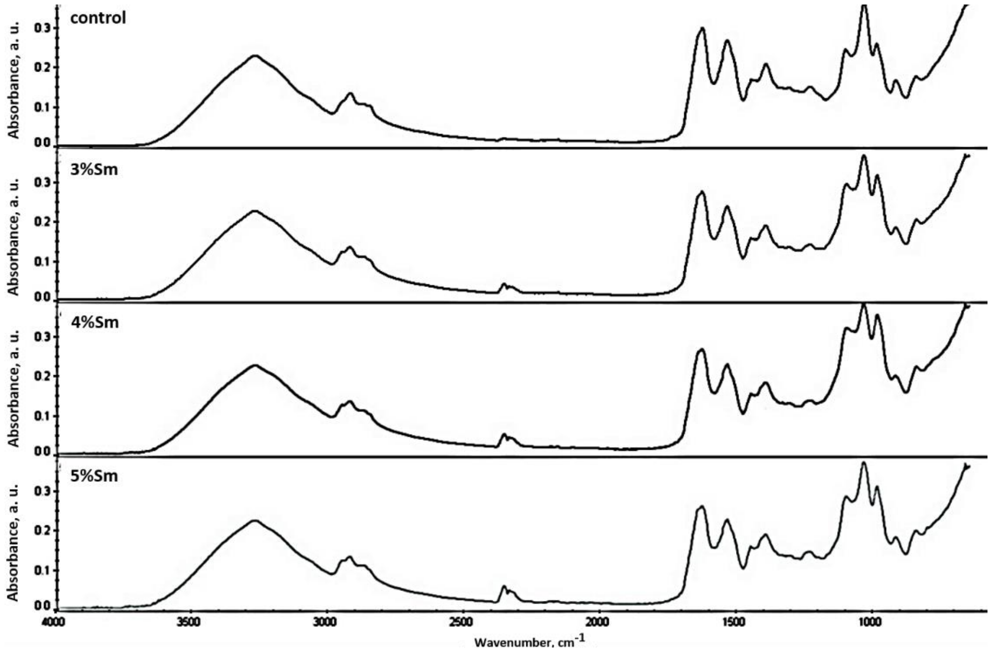

*Figure 4. FTIR spectra of PuOC-based films with the Satureja montana essential oil*

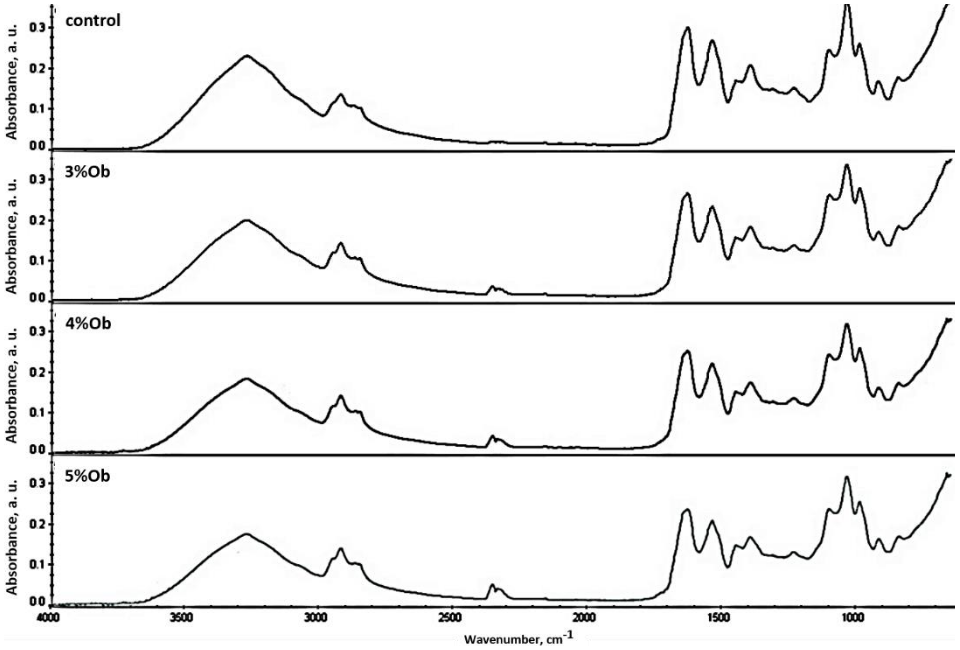

*Figure-5. FTIR spectra of PuOC-based films with the Ocimum basilicum essential oil*

The obtained peaks are associated to the O-H and N-H bond stretching vibrations [47]; in the 3000-2800 cm$^{-1}$ region, the peaks represent absorption of the C-H bond stretching [48]; the absorption bands present between the wavenumbers 1600 and 1700 cm$^{-1}$ represent the bands from amide I, that originates from the stretching vibration of the C = O bond (70–85 %) and is directly related to the conformation of the primary structure and amide II that originates from the N-H bond binding vibration (40-60 %) and the C-N bond stretching vibration (18-40 %) [49]. Amide I and amide

II are the two major areas of the infrared spectrum of proteins. In the 1200-1300 cm$^{-1}$ region the obtained peaks correspond to the amide III, derived from vibrations in C – N and N-H bond stretching, or vibrations of $CH_2$ groups [39,43,29]. The peak appearing in the wavenumber region from 1030 to 1045 cm$^{-1}$ was found in the obtained spectra of all film samples, corresponding to the OH group, mainly from glycerol that is used as a plasticizer [48,39].

Addition of essential oils into PuOC-based film, in general, did not induce significant changes regarding characteristic peaks as compared to the spectrum of the control film. Spectra of PuOC-based films with added essential oils showed similar major peaks, with somewhat different amplitudes in some cases. The largest decrement in the peak amplitude upon incorporation of the essential oils, was observed in the 3500-3000 cm$^{-1}$ region, being larger at higher oil concentrations. As it was mentioned previously, this region corresponds to the O-H and N-H bond stretching vibrations. Arrieta *et al.* [51] also reported a lower intensity of the peak at 3300 cm$^{-1}$ with the addition of carvacrol to sodium and calcium caseinate films, suggesting that those results present the reduction in the hydrophilic character of the films. Higher peak amplitude reduction was observed with incorporation of the *Ocimum basilicum* essential oil as compared to the *Satureja montana* essential oil, suggesting higher hydrophobic character of the films in the former case. These results are in correlation with the determined physicochemical properties of the films, where films with the *Ocimum basilicum* essential oil showed a slightly lower water and moisture sensitivity, as compared to the films with the other oil. However, with incorporation of the oils into the films, the increase in the intensity of the peak in the 3000-2800 cm$^{-1}$ region was observed, corresponding to the methylene asymmetrical and symmetrical stretching vibrations of the aliphatic C–H in $CH_2$ and $CH_3$ groups [29,44], respectively, and being more pronounced as the essential oil concentration was increased. Similar observations were reported previously [32,46] after incorporation of oregano essential oil into a gelatin-chitosan based film and citrus essential oil into gelatin film, suggesting that those bands are obviously present in most lipids [29,50]. Therefore, the addition of essential oils increased hydrophobic groups in the films, which could lead to an increase in the film hydrophobicity and a decrease in the WVTR.

With the addition of essential oils, a reduction of the peak in the 1100-1000 cm$^{-1}$ region, corresponding to the OH group from glycerol was also observed in a concentration dependent manner. Tongnuanchan *et al*. [32] made a similar observation suggesting that the amplitude of this peak decreased due to the dilution effect caused by the essential oil addition.

## 4. CONCLUSION

The results obtained in this study suggest that the hydrophobic character of *Satureja montana* and *Ocimum basilicum* essential oils influenced the properties of PuOC-based films after oil incorporation. In specific, lower water sensitivity and improved-barrier properties to water vapor and light transmission of the novel films, were observed. Further research should include examination of the material bioactivity (antioxidant and antimicrobial), and experiments using real food products. In this way, the influence of added active components on activity of PuOC-based material, as well as the influence of the material on the food product quality, shelf life and sensory properties, could be examined.

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## SAŽETAK

### Dodatak etarskih ulja u filmove na bazi pogače uljane tikve golice u cilju poboljšanja njihovih svojstava i osetljivosti na vodu

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(Naučni rad)

Biopolimerni materijali predstavljaju dobru alternativu za sintetske materijale. Međutim, njihova velika osetljivost prema vodi može da ograničava njihovu upotrebu, posebno za pakovanje proizvoda sa velikim sadržajem vode. Dodatak hidrofobnih komponenti u njihovu formulaciju, kao što su etarska ulja, može poboljšati ove osobine. U radu su sintetisani kompozitni biopolimerni filmovi na bazi pogače uljane tikve golice sa dodatkom 3, 4 i 5 % (v/v) *Satureja montana* ili *Ocimum basilicum* etarskih ulja, i ispitane su njihove mehaničke, fizičko-hemijske, barijerne i strukturne osobine. Dodatak etarskih ulja povećao je debljinu filma u odnosu na kontrolni film (film na bazi pogače uljane tikve golice bez dodatka etarskih ulja), ali je primećeno značajno smanjenje osetljivosti filmova na vlagu, povezano sa fizičko-hemijskim svojstvima i barijerom prema vodenoj pari (skoro 30 %), kao i poboljšanje barijere prema UV (transmisija manja od 1 %) i vidljivoj svetlosti (smanjenje transmisije oko 50 %) ($p < 0.05$). Dobijeni FTIR spektri su potvrdili prisustvo dodatih etarskih ulja, kao i njihov uticaj na smanjenje površinske hidrofilnosti filma. Pored značajnog doprinosa, primećeno je i pogoršanje mehaničkih svojstva ($p < 0.05$).

*Ključne reči*: biopolimerni materijali; lipidi; mehaničke osobine; fizičko-hemijske osobine; barijerne osobine; FTIR