INCORPORATION OF ESSENTIAL OILS INTO BIOPOLYMER FILMS BASED ON PUMPKIN OIL CAKE IN ORDER TO IMPROVE THEIR ANTIOXIDANT ACTIVITY

DODATAK ETARSKIH ULJA U BIOPOLIMERNI FILM NA BAZI POGAČE ULJANE TIKVE GOLICE U CILJU POBOLJŠANJA NJEGOVE ANTIOKSIDATIVNE AKTIVNOSTI

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ABSTRACT

Active films based on pumpkin oil cake, with 1 % and 2 % of winter savory and basil essential oil, were synthesized in the paper, and the antioxidant activity of the films obtained was subsequently examined. A biopolymer film based on pumpkin oil cake without the addition of essential oils was used as a control. The following methods were used for determining the antioxidant activity of the films: the DPPH radical scavenging activity, the hydroxyl radical scavenging activity and the reducing power assay. The control film showed some antioxidant activity (DPPH radical scavenging activity = 61 %, hydroxyl radical scavenging activity = 33 %, reducing power = 0.534). However, the addition of essential oils increased the antioxidant activity of the films, especially their reducing power, where as the film with 2 % of winter savory essential oil exhibited the highest activity. A slight increase in the DPPH radical and the hydroxyl radical scavenging activities was also observed with the incorporation of essential oils into the experimental films.

Key words: biopolymer films, pumpkin oil cake, essential oils, winter savory, basil, antioxidant activity

REZIME

Biopolimerni ambalažni materijali mogu poslužiti kao matriks za različite aktivne supstance, kao što su antioksidanti ili antimikotici. Mnoga etarska ulja karakterišu antioksidativna svojstva, pa je njihova upotreba kao prirodni antioksidansi od sve većeg značaja. Inkorporiranje etarskih ulja i drugih agenasa u aktivne filmove i premaze predstavlja odličnu alternativu direktnom inkorporiranju agenasa u namirnice, jer se na ovaj način, tokom određenog vremena, može omogućiti postepeno oslobađanje aktivnih komponenata iz ambalažnog materijala u upakovani proizvod, i tako produžiti rok trajanja proizvoda.

U radu su sintetisani aktivni filmovi na bazi pogače uljane tikve golice sa 1% i 2% etarskog ulja rtanjskog čaja i bosiljka, i ispitana je njihova antioksidativna aktivnost. Biopolimerni film na bazi pogače uljane tikve golice bez dodatnih etarskih ulja, korišćen je kao kontrola. Metode upotrebljene za određivanje antioksidativne aktivnosti filmova su: sposobnost hvatanja DPPH radikala, hidroksil radikala i određivanje redukcione moći. Na osnovu dobijenih rezultata, zapaža se da je kontrolni film pokazao određenu antioksidativnu aktivnost (sposobnost hvatanja DPPH radikala = 61%, sposobnost hvatanja hidroksil radikala = 33%, redukciona moć = 0,534), ali je dodatak etarskih ulja doveo do povećanja antioksidativne aktivnosti kontrolnog filma, posredno redukcione moći, gde je film sa 2% etarskog ulja rtanjskog čaja pokazao najveću aktivnost. U slučaju sposobnosti hvatanja DPPH radikala i hidroksil radikala, zapaženo je malo povećanje antioksidativne aktivnosti sa dodatkom etarskih ulja.

Ključne reči: biopolimerni filmovi, pogača uljane tikve golice, etarska ulja, rtanjski čaj, bosiljak, antioksidativna aktivnost.

INTRODUCTION

Synthetic plastic materials are widely used in all branches of industry, especially in packaging (where they are regarded as leading materials). This is primarily due to their good physical, mechanical and barrier properties, as well as their great suitability for processing, various possibilities of combining different materials with specific characteristics, and relatively low prices. However, these materials are non-renewable and non-degradable with a long life cycle. This has led to a major ecological problem of disposing and recycling synthetic packaging material waste. Therefore, in recent years, science has been increasingly focused on the use of biopolymer films (Lopez-Rubio *et al.*, 2006; Siracusa *et al.*, 2008; Lazić and Popović, 2015; Ribeiro-Santos *et al.*, 2017). The most common compounds for the formulation of biodegradable materials are polysaccharides, proteins and lipids, especially biopolymers obtained from agro-industrial waste (Popović *et al.*, 2015; Bulut *et al.*, 2017; Šuput *et al.*, 2018). Aimed at reducing the use of

chemical additives in the food industry, natural food additives are increasingly used at present. They are incorporated into biopolymer packaging materials and thus are gradually released into the food packaged therein, leading to an increase in the shelf life of food products (Ruiz-Navajas *et al.*, 2013). Essential oils are volatile, natural, complex compounds, formed from aromatic plants as secondary metabolites. They exhibit strong antioxidant and antimicrobial activity. The addition of essential oils to bio-based films and coatings, in order to obtain active packaging materials, may represent an effective method for preventing food oxidation and prolonging the shelf life of products (Aloui and Khwaldia, 2016; Atarés and Chiralt, 2016).

Owing to its chemical composition, pumpkin oil cake (*Cucurbita pepo* L. c.v. Olinka), a residual byproduct of pumpkin seed oil extraction, represents a potential raw material for obtaining biodegradable composite films (Popović *et al.*, 2011). Pumpkin oil cake (PuOC) contains 63 % of proteins, 12 % of carbohydrates, 4.5 % of crude fibers, 8.4 % of oils and 13 % of other components (Peričin *et al.*, 2007).

The experiment conducted involved incorporating two different essential aromatic oils, both belong to the *Lamiaceae* family, into pumpkin oil cake (PuOC) biopolymer-based films. The aromatic plant *Satureja montana* L. is commonly known as winter savory, and can be found at Mt. Rtanj in Serbia. The most dominant phenolic compounds in the essential oil of *Satureja montana*, responsible for its biological properties (antioxidant, antibacterial and fungicidal activity), are thymol and carvacrol (Vladić et al., 2016). The aromatic plant *Ocimum basilicum* L., commonly known as sweet basil, has antimicrobial, fungistatic and antioxidant activities due to its phenolic and aromatic compounds (Baritau et al., 1992). Linalool and methyl chavicol (estragole) were identified as two major components of basil essential oil, as well as eugenol (Filip et al., 2016).

The purpose of this study is to examine the effect of winter savory (*Satureja montana*) and basil (*Ocimum basilicum*) essential oils, at two different concentrations (namely 1% (v/v) and 2% (v/v)), on the antioxidant activity of active films based on PuOC. A film without essential oils was used as a control. In addition to the antioxidant activity of the experimental films, the activity of free essential oils (at concentrations of 1% (v/v) and 2% (v/v)) was also analyzed. The methods used to determine the antioxidant activity of the films were the DPPH radical scavenging activity, the hydroxyl radical scavenging activity and the reducing power assay.

MATERIAL AND METHOD

The grounded hull-less PuOC was kindly provided by *Linum* d.o.o., Čonoplja, Serbia and stored at a temperature of 4 °C. Winter savory (*Satureja montana*) essential oil and basil (*Ocimum basilicum*) essential oil were kindly supplied by the *Institute of Field and Vegetable Crops*, Novi Sad, Serbia. Other reagents used in this study were of analytical grade.

Preparation of active films

Active biofilms based on PuOC were prepared using the casting method. The film-forming suspension of PuOC (10 %, w/w) in deionized water was produced with the addition of 30 % glycerol (w/w, per weight of PuOC) and 0.2 % of guar-xanthan (w/w, per weight of polysaccharides in PuOC). After adjusting pH = 10 of the film-forming suspension, using 0.2 M NaOH, the suspension was incubated at 60 °C for 20 min and then filtered through a nylon filter. Winter savory or basil essential oils were added to the filtrate obtained (at concentrations of 1% (v/v) and 2% (v/v) respectively), and homogenized using an ultra-turrax homogenizer for 2 min at 20 000 rpm. The emulsions were cast onto Teflon-coated Petri dishes and the films were dried for 2 days, under room conditions (23 ± 2 °C, 50 ± 5 % RH). The films obtained were labeled as follows:

- 1wsPuOC - PuOC film with 1 % (v/v) of winter savory essential oil,
- 2wsPuOC - PuOC film with 2 % (v/v) of winter savory essential oil,
- 1bPuOC - PuOC film with 1 % (v/v) of basil essential oil,
- 2bPuOC - PuOC film with 2 % (v/v) of basil essential oil and
- control film without essential oils.

To measure the antioxidant activity of free essential oils, they were dissolved in ethanol to concentrations of 1% (v/v) and 2% (v/v), and labeled as follows:

- 1ws – 1 % of winter savory essential oil in an ethanolic solution,

- 2ws - 2 % of winter savory essential oil in an ethanolic solution,
- 1b – 1 % of basil essential oil in an ethanolic solution and
- 2b – 2 % of basil essential oil in an ethanolic solution.

Antioxidant activity methods

DPPH radical scavenging activity

The DPPH radical scavenging activity of the active films was estimated according to the procedure reported by Morales and Jimenez-Perez (2001). The films were cut in pieces (10x10 mm) and weighed (0.0195 ± 0.0019) g. To analyze the antioxidant activity of free essential oils, a total of 100µL of the prepared ethanolic solution was used. The samples were mixed with 4.8mL of the DPPH ethanolic solution (80 µM) and left on a shaker for 24h. The absorbance values were measured at 515 nm (JENWAY 6300, UK) and the DPPH radical scavenging activity was calculated by the following equation (Eq. 1):

$$\text{DPPH radi. scav. acti.} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \quad (1)$$

where A_{blank} is the absorbance value at 515 nm of the ethanolic solution of DPPH without the added sample (for films), or 100µL distilled water (for free essential oils), and A_{sample} is the absorbance value at 515 nm of the ethanolic solution of DPPH with the added sample.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity testing was performed according to Deeseenthum and Pejovic (2010). The films obtained were cut in pieces (5x5 mm) and weighed (0.0052 ± 0.0003) g. To analyze the antioxidant activity of free essential oils, 100µL of the prepared ethanolic solution was used. Every sample was mixed with 0.9 mL of sodium phosphate buffer (0.2 M, pH=7), 0.3 mL of 2-deoxyribose (10 mM), 0.3 mL of EDTA (10 mM), 0.3 mL of FeSO₄ (10 mM), 0.3 mL of hydrogen peroxide (10 mM) and 1 mL of distilled water. The samples were then incubated at 37 °C for 1 h. After incubation, 1.5 mL of trichloroacetic acid (2.8%) and thiobarbituric acid (0.1%) were added, and the samples were kept in a water bath for 10 min. After cooling, the samples were centrifuged at 3000 rpm for 10 min. The absorbance of each sample was measured at 520 nm (JENWAY 6300, UK). The antioxidant activity was calculated based on the following equation

$$\text{Hydroxyl rad. scav. acti.} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \quad (2)$$

where A_{blank} is the absorbance value at 520 nm of the reaction solution without the sample (for films), or 100µL distilled water (for free essential oils), and A_{sample} is the absorbance value at 520 nm of the reaction solution with the sample.

Reducing power assay

The reducing power of the films was determined according to Moradi et al. (2012). The films were cut in pieces (10x10 mm) and weighed (0.0212 ± 0.0014 g). To analyze the antioxidant activity of free essential oils, 100µL of the prepared ethanolic solution was used. Every sample was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50°C for 3 h. After incubation, 2.5 mL of trichloroacetic acid (100 g/L) was added to the mixture, which was then centrifuged at 3000 rpm,

for 10 min. The obtained supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of FeCl_3 (1 g/L) and the absorbance was measured at 700nm (JENWAY 6300, UK). An increase in the absorbance of the reaction mixture indicates an increase in the reducing power.

RESULTS AND DISCUSSION

The DPPH radical scavenging activity is the most commonly used method for determining the antioxidant activity of biopolymer-based films. It is an available, quick, simple and inexpensive means to measure the antioxidant activity. The antioxidant activity values of the films and essential oils were expressed as the percentage of free radical-scavenging activity (Figure 1). According to the results obtained, the control film showed some DPPH radical scavenging activity. Popović et al. (2010) already argued the antioxidant activity of PuOC films without incorporating any active substances. In comparison with the literature data on the antioxidant activity of other biopolymer-based films (such as biopolymer film based on hake protein, k-carrageenan, quince seed mucilage films, fish protein, carboxymethyl cellulose and chitosan films (Pires et al., 2013; Shojaei-Aliabadia et al., 2013; Jouki et al., 2014; Teixeira et al., 2014; Dashipour et al., 2015; Hafsa et al., 2016)) that showed the DPPH radical scavenging activity lower than 20%, the control film showed much higher activity (DPPH radical scavenging activity = 61%). The addition of 1% and 2% winter savory or basil essential oils slightly increased the scavenging activity of the control film. However, the influence of different concentrations of essential oils on the film antioxidant activity was not observed. Free essential oils showed higher antioxidant activity compared to active films at the same concentration of essential oils.

Fig. 1. DPPH radical scavenging activity of biopolymer active films and essential oils

The hydroxyl radical is highly reactive. It can be generated in food systems and may be important in initiating oxidative changes in foods (Korycka-Dahl and Richardson, 1980). Taking into account that the DPPH radical is a synthetic radical, the hydroxyl radical could be considered of greater importance than the DPPH radical, which is widely used for the determination of radical scavenging activity. However, this method is not commonly used for determining the antioxidant activity of

biopolymer-based films, probably because their short lifespan and high reactivity interfere with their direct detection (Xiang et al., 2011). The antioxidant activity of the films was expressed as the percentage of hydroxyl radical-scavenging activity (Figure 2).

Fig. 2. Hydroxyl radical scavenging activity of biopolymer active films

The activity values obtained for free essential oils were not displayed for all the concentrations measured because the negative results were obtained. This could indicate the pro-oxidative effect of the tested essential oils at certain concentrations. The pro-oxidative effect of linalool present in basil essential oil was already reported in the literature (Ruberto and Baratta, 2000), as well as the pro-oxidative effect of other different phenols (Ohshima et al., 1998; Santos et al., 2004). In addition, it has been reported that it is not uncommon for phenolic compounds to show both antioxidant and pro-oxidant activities depending on the doses applied (Llana-Ruiz-Cabello et al., 2015). Llana-Ruiz-Cabello et al. (2015) described that carvacrol and thymol, the main phenolic components in winter savory essential oil, could induce oxidative stress at higher concentrations. However, at lower concentrations, they play a protective role against oxidative stress. According to the results obtained, the control film showed some activity. However, with the incorporation of essential oils, the scavenging activity of PuOC based films increased. The films with winter savory essential oil showed higher activity compared to the films with basil essential oil, regardless of the concentration level. The reducing power of biopolymer active films and essential oils is presented in Figure 3. According to the results obtained, the control film showed a reducing power of 0.534. Compared to the data found in the literature, biopolymer films based on alginate and chitosan, without active compounds, showed a power lower than 0.500 (Norajitet et al., 2010; Moradi et al., 2012). With the addition of essential oils, the reducing power of the films significantly increased. The films with winter savory essential oil showed higher activity compared to films with basil essential oil. An increase in the antioxidant activity was recorded with increasing essential oil concentrations. Free winter savory essential oil showed higher values compared to the active films with the same concentration, which was not the case with basil essential oil. Teixeira et al. (2014) reported similar observations with garlic essential oil.

Fig.3. Reducing power of biopolymer active films and essential oils

CONCLUSION

Biopolymer films based on PuOC showed moderate antioxidant activity according to all the measuring methods employed, which proved higher than the antioxidant activity of other biopolymer-based films reported in the literature.

The incorporation of winter savory and basil essential oils into the PuOC films increased their antioxidant activity. The greatest improvement was observed in the reducing power of the films, especially with the addition of winter savory essential oil, whereas slight improvements were recorded in the radical scavenging activity of the films (DPPH and hydroxyl radical). An increase in the essential oil concentration increased the antioxidant activity of the PuOC based films. The results obtained indicate that the PuOC-based films with winter savory and basil essential oils can be used as active film. A further increase in essential oil concentrations would most likely result in a higher antioxidant activity of the films. However, it is necessary to devote particular attention to the proper concentration of essential oils added, that will not affect the organoleptic properties of the packaged products. This issue requires further research and development.

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