Investigation of a product-specific active packaging material based on chitosan biofilm with spice oleoresins

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Summary

In this paper, active edible packaging material was prepared and designed for potential application in sausage preservation. Material was prepared from chitosan with the addition of four oleoresins: garlic, black pepper, caraway and cinnamon. Antioxidative and antimicrobial activities of prepared films were tested and the film with optimal activity was further analysed as a food-packaging material. Chitosan film with added four oleoresins at a volume concentration of 2% (0.5% garlic, 0.5% black pepper, 0.5% caraway and 0.5% cinnamon) was found to be an effective antioxidant and antimicrobial material. Tensile strength and elongation at break showed weakening of the chitosan film with the addition of oleoresins. Water sensitivity of the chitosan film was lowered with oleoresins addition, considering film water content and swelling ability. Similar to pure chitosan film, chitosan film with oleoresins had very good barrier properties to oxygen and air, which could be compared to commercial barrier and high barrier films. The high water vapour permeability of the chitosan film was not decreased with the addition of oleoresins. Physical-mechanical properties and water vapour-barrier properties stay main possible drawbacks in application of chitosan film with oleoresins as self-standing packaging material, but its application would be possible as active coating and in combination with compatible materials.

Keywords

chitosan film; spice oleoresins; activity; packaging material

Increasing consumer demand for food free of chemical preservatives has led to the emergence of a large number of studies aimed at finding natural substances for use in food preservation. In this context, natural substances with pronounced antioxidative and antimicrobial properties, such as plant extracts and essential oils, gain in importance. Concentrated plant extracts (oleoresins) and essential oils can be considered as natural alternative in food preservation and their usage is in accordance with consumers' orientation to natural food with minimal chemical treatments [1]. Some of the most important spice herbs in meat industry in Serbia and the region are paprika, caraway, garlic and onion, black pepper, coriander, marjoram, mustard, cloves, ginger, cardamom, nutmeg, mace and cinnamon. Two main limitations in the usage of plant extracts and essential oils in food industry as preservatives are their pronounced sensory properties, which can affect acceptance of foods by consumers and loss of activity due to interaction with food components [2, 3].

On the other hand, packaging industry is searching for solution of the open question of packaging waste disposal, biopolymers being the possible development direction. Biopolymer films are natural materials from renewable sources. They degrade to carbon dioxide, water and methane or biomass in a comparatively short period, under the influence of oxygen, microorganisms and/or enzymes. Hydrocolloids, proteins and polysaccharides are molecules with pronounced filmogenic characteristics and have wide application in biopolymer film production. Hydro-

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colloid films have good barrier properties to gases and lipids, good mechanical properties and do not affect sensory properties of the packed product (food or medicine) [4]. Chitosan is a filmogenic polysaccharide obtained from the food industry waste. It is approved as a food additive in many countries. This polysaccharide possesses three functional groups that allow different molecular modifications. It acts as an antimicrobial and antioxidative agent and forms films with good barrier properties for gases and good mechanical properties. All these properties indicate the possibility of chitosan usage for natural edible biodegradable active packaging material production [5–7].

Chitosan may act as a carrier matrix for gradual release of active substances of plant origin, which can increase the activity of the biopolymer and slow down evaporation of the active substance. Two main limitations in oleoresins application as preservatives could be prevented using oleoresins of the common spices in packed food production (where pronounced organoleptic properties would be advantageous) and by gradual release from the chitosan matrix (which would compensate for the loss of activity due to interaction with food). Packaging based on biopolymers with the addition of plant extracts or essential oils are, apart from its basic function to contain and protect food until consumption, active, natural and biodegradable [8–10].

In this paper, active edible packaging material was prepared, designed for potential application in sausage preservation. Material was prepared from chitosan with the addition of four oleoresins frequently used in sausage production in Serbia and the region, namely, garlic, black pepper, caraway and cinnamon. Antioxidative and antimicrobial activities of the prepared films were tested and the film with optimal activity was further analysed for its most important properties regarding its application as a food packaging material.

MATERIALS AND METHODS

Reagents

Commercial chitosan powder from crab shells, highly viscous (deacetylation degree approx. 80%) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemical (St. Louis, Missouri, USA). Four oleoresins for food industry – cinnamon, black pepper, garlic and caraway were obtained from the local manufacturer of spices, additives and aromas, Milex (Novi Sad, Serbia). Tween 20 was purchased from Superlab (Belgrade, Serbia). And all other chemicals were of the highest grade commercially available, and all solutions were prepared using deionized, distilled water.

Film preparation

Chitosan film-forming solution was prepared by dissolving chitosan powder in 1% acetic acid to reach chitosan mass-per-volume ratio of 10 $kg \cdot m^{-3}$. Solution was left stirring overnight (20 h to 24 h) on a magnetic stirrer to dissolve chitosan. After dissolving chitosan, the solution was vacuum-filtrated to remove undissolved particles. Four oleoresins in equal volume concentrations and the wetting agent, Tween 20 (50% of total oleoresins volume), were added and the mixture was homogenized with Silent Crusher M (Heidolph, Chicago, Illinois, USA), using treatment of 333.34 Hz for 2 min, 2 min pause and again 333.34 Hz for 2 min, to obtain white homogenous emulsions. Emulsions were casted on Petri dishes covered with Teflon coating and left to air-dry (23 °C ± 3 °C, relative humidity, *RH*, 50% ± 10%) on a horizontal table surface. Films were labelled according to concentration, as:

- C – for chitosan film without oleoresins,
- O1 – for chitosan film with a mass-per-volume ratio of oleoresins of 8 $kg \cdot m^{-3}$: 2 $kg \cdot m^{-3}$ garlic, 2 $kg \cdot m^{-3}$ black pepper, 2 $kg \cdot m^{-3}$ caraway and 2 $kg \cdot m^{-3}$ cinnamon
- O2 – for chitosan film with a mass-per-volume ratio of oleoresins of 16 $kg \cdot m^{-3}$: 4 $kg \cdot m^{-3}$ garlic, 4 $kg \cdot m^{-3}$ black pepper, 4 $kg \cdot m^{-3}$ caraway and 4 $kg \cdot m^{-3}$ cinnamon
- O3 – for chitosan film with a mass-per-volume ratio of oleoresins of 24 $kg \cdot m^{-3}$: 6 $kg \cdot m^{-3}$ garlic, 6 $kg \cdot m^{-3}$ black pepper, 6 $kg \cdot m^{-3}$ caraway and 6 $kg \cdot m^{-3}$ cinnamon.

Antioxidant activity

The potential antioxidant activity of the films was assessed on the basis of scavenging the stable •DPPH free radical. The •DPPH scavenging activity of the films was determined according to Morales and Jimenez-Perez [11], with some modification. In brief, 100 mg of the film was placed in a flask containing 2.4 ml of daily prepared 0.16 $mmol \cdot l^{-1}$ ethanolic solution of •DPPH, and was stirred for 2.5 h, 4 h or 24 h, at room temperature. The controls were without the presence of films. In each sample, the remaining •DPPH concentration was determined after removing the solid film by measuring the absorbance at 520 nm using T80/T80+ UV-Vis spectrophotometer (PG Instruments, Lutterworth, United Kingdom), using the calibration curve, made with standard solutions of •DPPH radical. Antioxidant activity

of the films was expressed as a percentage and calculated following Eq. 1.

$$AA = \frac{(1 - c_s)}{c_c} \times 100 \quad (1)$$

where AA is antioxidant activity, c_s is the concentration of •DPPH in the tested sample and c_c is the concentration of •DPPH in the respective blank.

Antimicrobial activity

Antimicrobial activity was tested by ASTM E2149 method [12], which is a quantitative antimicrobial test method performed under dynamic contact conditions. Gram-positive (*Staphylococcus aureus* ATCC 25923; Microbiologics, St. Cloud, Minnesota, USA) and Gram-negative bacteria (*Escherichia coli* ATCC 8739; Microbiologics) were used as test organisms. Fresh inoculants for antibacterial assessment were prepared on nutrient agar (Merck, Darmstadt, Germany) at 37 °C for 24 h. The incubated test culture was diluted using a sterilized 3 mmol·l^{-1} phosphate buffer (KH_2PO_4; pH 6.8) to give a final concentration of (1.5×10^5)–(3×10^5) CFU·ml^{-1}. This solution was used as a working bacterial dilution.

Film samples were treated with UV lamp (Kruss, Hamburg, Germany) irradiation at a wavelength of 254 nm for 30 min for each side of the film. Then it was cut into test pieces, which had contact surface area of 58 cm^2, and transferred to a 250 ml Erlenmeyer flask containing 50 ml of the working bacterial dilution. All flasks were capped loosely and shaken for 24 h at room temperature and 2 Hz using a wrist action incubator shaker (shaker model 75-BB; Burrell Scientific, Pittsburgh, Pennsylvania, USA) and placed in an incubator (model IL-21; JEIO TECH, Seoul, Korea). At different contact periods, 3 h and 24 h, 1 ml of the bacterial dilution was withdrawn, serially diluted and plated on tryptone soya agar (Merck). The inoculated plates were incubated at 37 °C for 24 h, and colonies were counted. The average values of the duplicates were converted to colony forming units per millilitre in the flasks by multiplying with the dilution factor. The antimicrobial activity was expressed as percentage of reduction (R) of the organism after contact with the test specimen compared to the number of microorganisms cells surviving after contact with the control (Eq. 2).

$$R = \frac{(B - A)}{B} \times 100 \quad (2)$$

where A represents counts for the flask containing the treated substrate after the specified contact time and B represents "0" contact time counts for the flask used to determine A before the addition of the treated substrate (expressed in colony forming units per millilitre). Reduction R was calculated using average of 4 replicates for A and B.

After 24 h in bacterial solution, films were transferred from bacterial suspension onto Petri dishes with nutrient agar and incubated for 24 h at 37 °C to obtain further insight in the antimicrobial activity of the films.

Physical-mechanical properties

Film thickness was measured using a micrometer Digico 1, with sensitivity of 0.001 mm (Tesa, Renens, Switzerland). Eight thickness measurements were carried out. Tensile strength and elongation at break were measured on the Instron Universal Testing Instrument Model No 4301 (Instron Engineering, Canton, Massachusetts, USA), according to ASTM standard method D882-10 [13]. A rectangular film strip of 90 mm in length and 15 mm in width was used. The initial grip separation was set at 50 mm and crosshead speed was set at 12.5 mm·min^{-1}. Tensile strength and elongation at break measurements were repeated at least five times.

Physical-chemical properties

Water content

Film samples (2 cm × 2 cm) were conditioned in different starting atmospheres during 48 h. Used atmospheres had temperature (23 ± 3) °C and RH = 0%, RH = 50% or RH = 90%. Film samples were weighed (w_1), dried at 105 °C for 24 h and weighed again (w_2). Water content (WC) was determined as the percentage of initial film weight lost during drying and reported on wet basis (Eq. 3) [14].

$$WC = \frac{(w_1 - w_2)}{w_1} \times 100 \quad (3)$$

Triplicate measurements of WC were conducted.

Swelling ability

The films were cut into pieces of (1 cm × 2 cm) and weighed in air-dried conditions (w_1). Then they were immersed in deionized water (25 °C) for 2 min. Wet samples were wiped with filter paper to remove excess liquid and weighed (w_2). The amount of adsorbed water was calculated as shown in Eq. 4.

$$SW = \frac{(w_2 - w_1)}{w_1} \times 100 \quad (4)$$

where w_2 and w_1 were the weights of the wet and the air-dried samples, respectively [14]. Swelling

ability (*SW*) was also tested in buffers of pH 4, pH 7 and pH 9. Each measurement was repeated three times.

Total soluble matter

Small pieces of film (2 cm × 2 cm) were dried in the oven at 105 °C for 24 h to obtain the initial dry mass of the film. After drying, films were placed into test tubes containing 20 ml of deionized water. Test tubes were covered and gently shaken for 24 h at room temperature. The remaining pieces of film were dried in the oven at 105 °C for 24 h to obtain final dry mass of the film [14]. The percentage of total soluble matter (*TSM*) of the films was calculated using Eq. (5).

$$TSM = \frac{(w_1 - w_2)}{w_1} \times 100 \quad (5)$$

where w_1 is initial dry mass before the test and w_2 is final dry mass after the test.

Barrier properties

Permeability of gases

Permeability to oxygen, carbon dioxide and nitrogen were measured using the method of Lyssy, according to DIN 53 380 [15] on the device Lyssy GPM-200 (Systech Instruments, Thame, United Kingdom) with a gas chromatograph Gasukuro Kogyo GC-320 (GL Sciences, Tokyo, Japan) and HP 3396 integrator (Hewlett Packard, Palo Alto, California, USA). Permeability to air was numerically calculated. A minimum of three measurements were carried out.

Water vapour transmission rate

Moisture barrier properties of films were determined gravimetrically by the dish method according to ISO 2528:1995 [16], condition A (temperature 25 °C ± 1 °C and *RH* 90% ± 2%, obtained using a saturated solution of potassium nitrate). Silica gel was used as desiccant for filling the test dish. Three replicates were tested simultaneously.

Statistical analysis

Statistical analysis was carried out using OriginPro 8 (OriginLab, Northampton, Massachusetts, USA). All data were presented as mean values with their standard deviation indicated. Variance analysis (ANOVA) was performed with a confidence interval of 95% ($p < 0.05$). Means were compared by the Tukey test.

RESULTS AND DISCUSSION

Antioxidant activity

Data on antioxidant activity of tested films are presented in Fig. 1. Although the activity of pure chitosan film increases in time, from (9.3 ± 2.3)% to (25.1 ± 3.4)%, it might be considered as weak. Activity of chitosan molecule in •DPPH scavenging is based on the activity of free amino and hydroxyl groups, which can be donors of electron or hydrogen atom. Activity of chitosan film is known to be lower compared to chitosan solution, due to strong hydrogen bonds in the film that block active scavenging groups and depend on test duration [17–19]. Similar, or even lower values for chitosan film •DPPH scavenging activity can be found in literature [20–25]. To optimize antioxidant activity of chitosan film, different natural or synthetic active compounds of low molecular weight can be added into the film [20–28]. In this paper, four different oleoresins from spices commonly used in dry fermented sausage production were added as active compounds into the chitosan film. Addition of oleoresins at a mass-per-volume ratio of 8 kg·m^{-3} into the film resulted in a significant increase of its antioxidant activity ($p < 0.05$). Similar to chitosan film activity, activity of O1 film increased in time, from (46.9 ± 4.3)% to (94.0 ± 0.3)%. For O2 film, antioxidant activity was further increased, compared to O1 film, after 2.5 h and 4 h of test duration, while after 24 h, there was no significant difference between the activities of the two films. Further addition of

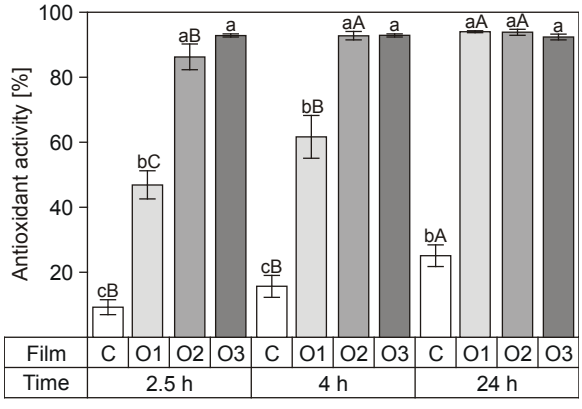

Fig. 1. Antioxidant activity of films.

Different lowercase letters mark significantly different mean values ($p < 0.05$) between all tested films after a specific period of time. Different uppercase letters mark significantly different mean values ($p < 0.05$) of a specific film after different periods of time.
C – chitosan film without oleoresins, O1 – chitosan film with 8 kg·m^{-3} of oleoresins, O2 – chitosan film with 16 kg·m^{-3} of oleoresins, O3 – chitosan film with 24 kg·m^{-3} of oleoresins.

oleoresins (film O3) caused no further increase in antioxidant activity, compared to O2 film. Based on the results shown in Fig. 1, it can be concluded that films O1 and O2 were appropriate for application as active antioxidant films.

Antimicrobial activity

Data on antimicrobial activity of chitosan films with and without oleoresins are presented in Tab. 1. Antimicrobial agent effectiveness, regarding the dynamic shaking test in accordance with the standard ASTM E 2149-01, is significant in practice if the reduction of microorganisms is higher than 75% [29]. Considering this requirement, it can be seen from Tab. 1 that chitosan films with and without oleoresins showed significant (> 75% reduction) antimicrobial activity against *E. coli* and *S. aureus*. For both *E. coli* and *S. aureus* reduction, time of contact had significant influence ($p < 0.05$). For an increased contact period, antimicrobial activity increased. When oleoresins were added into the chitosan film, its antimicrobial activity against *E. coli* was increased, for films O2 and O3 ($p < 0.05$), while activity against *S. aureus* was not improved by oleoresins addition.

For further analysis, after 24 h of incubation in bacterial solution, films were transferred to Petri dishes with nutrient agar and incubated for 24 h at 37 °C. It was expected that, if the film had bacteriostatic or bactericidal properties, there would be no growth of bacteria in contact with the film. On the other hand, if there was a bacteriostatic or bactericidal effect of incorporated oleoresins, there would be also inhibition of growth of bacteria around the film, due to diffusion of the active compound(s) from the film into the nutrient medium [5, 30–32]. Results of this prolonged qualitative test are shown in Fig. 2.

For both tested bacteria, when chitosan film was transferred from bacterial suspension onto nutrient agar, bacterial growth was detected under the film (in contact with the film), as well as around the film. This suggested that chitosan film did not show either bacteriostatic or bactericidal effect. These results seem to be contradictive to the results for reduction of bacterial number after 24 h (Tab. 1). Our hypothesis is that in bacterial suspension, reduction was high because cells were adsorbed onto the film surface as a result of chitosan polycationic nature, but this interaction did not damage the cells and they continued their growth after being transferred onto nutrient agar. Addition of oleoresins into the film improved its antimicrobial activity. For O2 and O3 films, no growth of both test bacteria was detected in contact with the film or in the film surrounding. This indicated bacteriostatic, as well as bactericidal effects of the films. Based on the analysis of antibacterial action against two test bacteria, films O2 and O3 can be considered as effective antimicrobial films. Taking into account results of both biological activity tests, film O2 was selected as appropriate film for active packaging film production and this film was used in further analysis of packaging material properties.

Physical-mechanical properties

Thickness, tensile strength and elongation at break of tested films were determined and obtained data are presented in Tab. 2. Thickness of chitosan film was significantly increased ($p < 0.05$) by the incorporation of oleoresins. Thickness of chitosan films was (67.67 ± 9.17) μm, while chitosan film with addition of oleoresins had thickness of (174.11 ± 24.73) μm. Both films were obtained by casting the same volume of filmogenic solution (emulsion) on Petri dish of the same surface. Thickness increase of chitosan film with the addition of different dissolved and emulsified substances, essential oils, green tea extract and grape seed extract was reported by Zivanovic et al. [33], Peng and Li [34] and Amanakwaah [35]. With

Tab. 1. Antimicrobial activity of films.

Microorganism	Contact period	Antimicrobial activity [%]			
		C	O1	O2	O3
E. coli	3 h	32.9 ± 5.9^{dC}	35.7 ± 12.0^{dB}	74.4 ± 2.4^{cB}	91.5 ± 5.8^{abA}
	24 h	94.9 ± 3.8^{abA}	87.0 ± 4.4^{bA}	99.9 ± 0.0^{aA}	99.9 ± 0.0^{aA}
S. aureus	3 h	95.2 ± 9.4^{aA}	37.3 ± 13.1^{cB}	65.3 ± 10.3^{bB}	99.2 ± 0.3^{aA}
	24 h	99.9 ± 0.0^{aA}	76.7 ± 10.2^{bA}	99.9 ± 0.0^{aA}	99.2 ± 0.3^{aA}

Antimicrobial activity is expressed as percentage of reduction.
Different lowercase letters mark significantly different mean values ($p < 0.05$) within the same row. Different uppercase letters mark significantly different mean values ($p < 0.05$) within the same column.
C – chitosan film without oleoresins, O1 – chitosan film with 8 kg·m^{-3} of oleoresins, O2 – chitosan film with 16 kg·m^{-3} of oleoresins, O3 – chitosan film with 24 kg·m^{-3} of oleoresins.

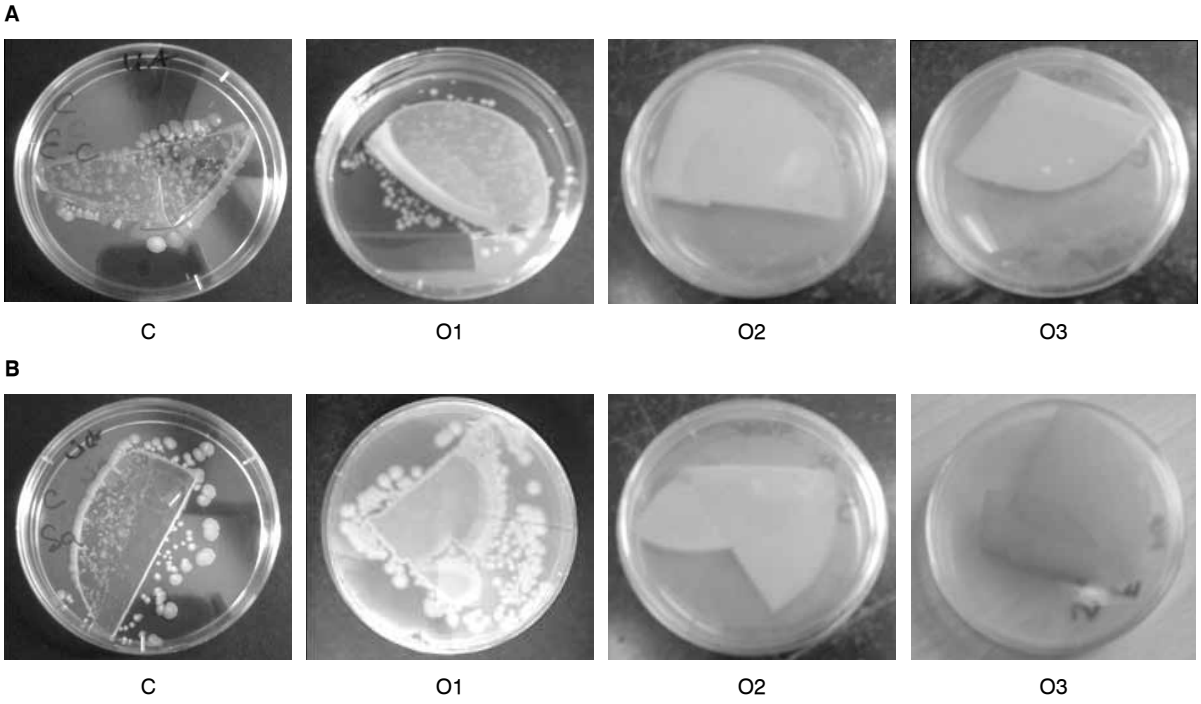

Fig. 2. Prolonged antimicrobial test.

A – films from *E. coli* suspension, B – films from *S. aureus* suspension.
C – chitosan film without oleoresins, O1 – chitosan film with 8 kg·m^{-3} of oleoresins, O2 – chitosan film with 16 kg·m^{-3} of oleoresins, O3 – chitosan film with 24 kg·m^{-3} of oleoresins.

the addition of low molecular weight compounds into the film (essential oils, extracts), molecular interaction between chitosan molecules and added compound increases, while aggregation interactions between chitosan molecules weaken, resulting in a more open polymeric matrix and in thicker films [36].

The determined tensile strength and elongation at break of chitosan film, (67.67 ± 9.17) MPa and (19.1 ± 5.7)%, were in accordance with literature data for tensile properties of unplasticized chitosan films casted from acetic acid solution [5, 37]. Tensile properties presented in Tab. 2 indicate weakening of chitosan film with the addition of oleoresins into the film. From Tab. 2 it is evident that tensile strength of chitosan film decreased with the addition of oleoresins, the decrease of tensile strength being 82%. Results are in accordance with literature data where incorporation of active compounds without cross-linking function, such as essential oils, carvacrol, potassium sorbate, nisin, green tea and grape seed extracts or oleic acid into the chitosan, led to a decrease in tensile strength of the film [28, 35, 38–41]. It was assumed that aggregation forces between chitosan molecules are weakened by the contact between chitosan molecule and incorporated compounds. Also, hydrophobic compound in the film structure causes discontinuities, as lipids do not form continuous cohesive matrix, and lower water content in the film, which all leads to a decrease in mechanical resistance [28, 36, 40, 42–44]. Different results were reported by Peng and Lee [34], Rubilar et al. [45] and Ojagh et al. [46], where tensile strength of chitosan films did not change or even increased with the addition of essential oils. Considering that tensile strength is in correlation with the microstructure of the polymer matrix and intermolecular forces within the matrix, it is possible that tensile strength varies depending on the type of active compound added, on properties of

Tab. 2. Physical-mechanical properties of films.

	Film	
	C	O2
Thickness [μm]	67.67 ± 9.17^{b}	174.11 ± 24.73^{a}
Tensile strength [MPa]	78.62 ± 6.35^{a}	14.18 ± 5.15^{b}
Elongation at break [%]	19.1 ± 5.7^{a}	3.6 ± 0.8^{b}

Different lowercase letters in superscript mark significantly different mean values ($p < 0.05$) within the same row.
C – chitosan film without oleoresins, O2 – chitosan film with 16 kg·m^{-3} of oleoresins.

Tab. 3. Physical-chemical properties of films.

	Test conditions	Film	
		C	O2
Water content [%]	*RH* 0%	12.9 ± 4.2^{C}	10.0 ± 1.3^{C}
	RH 50%	19.2 ± 1.9^{aB}	12.8 ± 0.4^{bB}
	RH 90%	42.2 ± 0.9^{aA}	24.6 ± 1.1^{bA}
Swelling ability [%]	Water	853.0 ± 4.6^{a}	83.6 ± 1.1^{b}
	pH 4	797.5 ± 80.7^{a}	77.4 ± 4.9^{b}
	pH 7	718.7 ± 68.0^{a}	78.5 ± 1.2^{b}
	pH 9	741.4 ± 62.6^{a}	84.7 ± 2.5^{b}
Total soluble matter [%]		7.6 ± 2.1	9.3 ± 1.1

Different lowercase letters in superscript mark significantly different mean values ($p < 0.05$) within the same row. Different uppercase letters in superscript mark significantly different mean values ($p < 0.05$) within the same column.
RH – relative humidity, C – chitosan film without oleoresins, O2 – chitosan film with 16 kg·m^{-3} of oleoresins.

chitosan molecule, on plasticizer addition and/or emulgation conditions [47].

Values for chitosan film elongation at break decreased with the addition of oleoresins. Ojagh et al. [46] and Sánchez-González et al. [48] also reported a decrease of elongation at break when essential oils were added into hydrocolloid film. Different results were presented by Pranoto et al. [32], Krkić et al. [38] and Benavides et al. [49], who reported that addition of essential oils into hydrocolloid films did not affect elongation at break of the film. However, Chi [39], Bonilla et al. [36] and Amanakwaah [35] reported an increase of the elongation at break values for the biofilms with added essential oils. Addition of low molecular weight components into the filmogenic solution affects intermolecular cohesive forces between macromolecules, as previously mentioned. Depending on the type of the low molecular weight compound, its concentration, energy of mixing and other factors, three cases are possible: plasticizing effect, weakening of the film or no change in film elasticity.

Comparing chitosan film tensile properties with these properties of most frequently used polymer films reported by Crompton [50], it can be seen that chitosan film tensile strength value was higher than the value reported for high density polyethylene (HDPE), low density polyethylene (LDPE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) or polyamides (PA) except PA 4,6, while chitosan film with added oleoresins had tensile strength that could be only compared with LDPE. When elongation at break of chitosan film with and without the addition of oleoresins is compared with values for commercially used polymer films reported by Crompton [50], it can be seen that chitosan film without oleoresins had elongation at break values that could be compared to a few commercial polymer films (PA 6,9; PA 4,6; PVC), while chitosan film with oleoresins had low values for this mechanical characteristic [50]. Comparison with commercial polymers is, however, only approximate and illustrative, as physical properties of polymers depend on numerous factors that are not specified in the given comparison.

Physical-chemical properties

Data on physical-chemical properties of chitosan film and chitosan films with added oleoresins are presented in Tab. 3. These characteristics are related to biofilm sensitivity to moisture. Hydrocolloid films are sensitive to moisture and this characteristic affects their application to a great extent. By the addition of lipophilic compounds into the film, it is possible to decrease sensitivity to moisture.

Water content in the film depended on atmospheric conditions. For every *RH* value in the atmosphere, there was a corresponding value of water content in the film and, as *RH* in the atmosphere increased, water content in the film increased accordingly ($p < 0.05$). In low *RH* conditions, physical binding of water molecules on hydroxyl groups in the polymer takes place only at the surface of the film. This leads to local dissolution of the film and swelling of the polymer film, which results in the release of new groups as active spots in the polymer. For intermediate *RH* values, water absorption also occurs in less accessible places, which leads to plasticization of the polymeric film. Plasticization of the film leads to the exposure of numerous active spots for water binding, which might result in decomposition of the polymeric film structure [51–53]. For low *RH* values ($RH = 0\%$), there was no difference in water contents between films with and without oleoresins, while for intermediate ($RH = 50\%$) and high *RH* values ($RH = 90\%$), water content was lower in the film with oleoresins ($p < 0.05$). This was probably due to interaction of oleoresins and chitosan molecule, where oleoresins were distributed in the polymer matrix in a manner that left a low number of free active groups for water binding [53].

Similar but more pronounced effect was recorded for film swelling ability, where addition of oleoresins led to a significant decrease (10-times reduction) of film swelling ability in all tested

media (water and different buffer solutions). Between different buffer solutions, there was no difference in swelling ability of films. This was probably because the films were not neutralized after production, leaving a certain amount of acetic acid entrapped in the film matrix.

Dissolution of chitosan film is a process that follows swelling of the film. Solubility is connected to protonation of $-NH_2$ group on C(2) position of D-glucosamine unit, which is followed by gradual transformation of polysaccharide in acidic media to polyelectrolyte. Addition of oleoresins into the chitosan biofilm did not influence solubility of chitosan film in water ($p > 0.05$).

Barrier properties

Oxygen and air transmission rates, as well as water vapour transmission rate and permeability are presented in Tab. 4. Gas permeability, especially oxygen permeability of packaging films, is very important for food shelf life, due to oxygen being the key factor in lipid oxidation and initiation of deteriorative reactions in food [54, 55]. Polysaccharide films are known to be agood oxygen barrier due to tight arrangement of molecules and formation of hydrogen bonds network [21, 45]. Oxygen permeability of chitosan film without the addition of oleoresins was $(4.56 \pm 0.94) \times 10^{-6}$ ml·m^{-2}h^{-1}Pa^{-1}, while addition of oleoresins caused an increase in oxygen permeability to $(9.40 \pm 1.59) \times 10^{-6}$ ml·m^{-2}h^{-1}Pa^{-1}($p < 0.05$). Similar results for chitosan film oxygen permeability were reported by ALTIOK et al. [28] and DI PIERRO et al. [56]. Increase in film oxygen permeability with the addition of oleoresins is in accordance with literature data, as it was reported that addition of a hydrophobic compound into the hydrocolloid film affected film continuity and increased its permeability [28, 45]. Comparing results presented in Tab. 4 to permeability of commercial polymer films used for food packaging, permeability of both chitosan film with and without oleoresins was in the range of barrier and high barrier films used for packaging in vacuum and in modified atmosphere [54, 55, 57].

Water vapour permeability of hydrophilic films depends on film solubility and water molecule diffusion through the film matrix [38, 58]. When a hydrophobic compound is added into the film, it might lead to weakening of intermolecular interactions between polymer molecules, resulting in an open structure and increased diffusivity. On the other hand, addition of a hydrophobic compound can decrease water vapour permeability by different mechanisms: increase in hydrophobic nature of the matrix, increased density of the matrix, decrease of interspaces between molecules and/or to a decreased number of active hydroxyl groups due to molecular interactions between the polymer and the hydrophobic compound [22, 23, 32, 35, 39]. Depending on which effect dominates in a specific case, water vapour permeability might increase or decrease with the addition of a lipophilic compound, or different effects might be balanced so that the permeability of the film stays unchanged. Also, MCHUGH et al. [59] showed positive correlation between film thickness and its water vapour permeability. High values of film thickness can affect relative humidity around the film, affecting further water absorption kinetics [59, 60].

Water vapour transmission rate is a parameter that excludes the effect of film thickness on vapour transmission. Addition of oleoresins into the film led to a decrease in water vapour transmission rate of chitosan film, from (8.91 ± 0.27) g·m^{-2}h^{-1} to (6.54 ± 0.79) g·m^{-2}h^{-1} . When the increase in the film thickness is taken into consideration, water vapour permeability values show that addition of oleoresins led to an increase in water vapour permeability, from $(5.82 \pm 0.17) \times 10^{-11}$ g·m^{-1}s^{-1}Pa^{-1} for chitosan film to $(10.99 \pm 0.13) \times 10^{-11}$ g·m^{-1}s^{-1}Pa^{-1} for chitosan film with oleoresins. Although this increase was statistically significant ($p < 0.05$), from the application point of view, it was not relevant. Literature data show that chitosan films, depending on molecular weight, deacetylation degree, type and concentration of plasticizer and synthesis process, have water vapour permeability that ranges from 3.66×10^{-11} g·m^{-1}s^{-1}Pa^{-1}

Tab. 4. Barrier properties of films.

	Film	
	C	O2
Oxygen transmission rate [ml·m^{-2}h^{-1}Pa^{-1}]	$(4.56 \pm 0.94) \times 10^{-6}$ ^{b}	$(9.40 \pm 1.59) \times 10^{-6}$ ^{a}
Air transmission rate [ml·m^{-2}h^{-1}Pa^{-1}]	$(2.72 \pm 1.64) \times 10^{-6}$ ^{b}	$(5.63 \pm 0.94) \times 10^{-6}$ ^{a}
Water vapour transmission rate [g·m^{-2}h^{-1}]	8.91 ± 0.27 ^{a}	6.54 ± 0.79 ^{b}

Different lowercase letters in superscript mark significantly different mean values ($p < 0.05$) within the same row.
C – chitosan film without oleoresins, O2 – chitosan film with 16 kg·m^{-3} of oleoresins.

to 15.27×10^{-11} $g \cdot m^{-1}s^{-1}Pa^{-1}$. Results in this paper are comparable to these values [22, 23, 35, 44]. Compared to other biopolymer films, chitosan films with and without oleoresins have lower water vapour permeability than maize starch, sweet potato starch, gelatine, carrageenan, wheat gluten, hydroxypropyl methylcellulose and some zein films, while methyl cellulose and some zein films have permeability comparable to present analysed films. Compared to commercial polymeric films, chitosan films with and without oleoresins have water vapour permeability that is comparable to cellophane films, which can be expected, considering structure similarities of these two polymers. However, this value for chitosan films is considerably (about hundred times) higher compared to LDPE and HDPE films [61, 62].

CONCLUSIONS

Chitosan film with added oleoresins of four frequently used spices in sausage production, namely, cinnamon, garlic, black pepper and caraway, was shown to be an effective antioxidant and antimicrobial material. Similar to pure chitosan film, chitosan film with oleoresins had very good barrier properties for oxygen and air, while, compared to pure chitosan film, film with oleoresins had lower sensitivity to moisture. Physical-mechanical properties and water vapour barrier properties stay main possible drawbacks in application of chitosan film with oleoresins as self-standing packaging material, but its application would be possible as active coating and in combination with compatible materials.

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