Application of chitosan coating with oregano essential oil on dry fermented sausage

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Summary

Chitosan film was produced from a highly viscous chitosan solution in acetic acid. Oregano essential oil was added to the film-forming solution, before casting it on a Petri dish. Film mechanical properties (thickness, tensile strength and elongation at break) and barrier properties (water vapour and oxygen permeability) were investigated. The film proved to be strong but brittle with good barrier properties to oxygen and poor barrier properties to water vapour. In second experiment, traditional dry fermented sausage "Petrovská klobása" (label of geographical origin) was coated with chitosan-oregano film-forming solution and stored in a chamber with controlled temperature (15 °C) and relative humidity (75%) for five months. Analyses of moisture content, colour, lipid oxidation as well as sensory analysis of taste and smell were conducted before coating and on 150th day of storage. Coating the sausage with chitosan film slowed down the moisture loss and retarded lipid oxidation, while there was no change in colour of the sausage. Coated sausage obtained better results from sensory evaluation of taste and smell. Results are encouraging for further optimization of natural active packaging as a way of preservation of "Petrovská klobása" sausage.

Keywords

chitosan; coating; oregano; sausage

Polysaccharide chitin (poly-β-(1 → 4)-*N*-acetyl-D-glucosamine) is widely abundant in nature. It is a major component of the insect exoskeletons, marine invertebrates and the cell walls of many fungi. Until now, chitin has been commercially produced from shellfish waste. Utilizing shellfish waste for chitin production provides a solution for the waste disposal problem [1].

Use of chitin is limited by its insolubility. It is insoluble in water, in common organic solvents and in acidic, alkali and neutral aqueous solutions [2].

Chitin can be deacetylated using a sodium hydroxide solution or with a chitin deacetylase enzyme to yield chitosan, a biopolymer that is soluble in organic acids [1, 2]. Chitosan (poly-β-(1, 4)-D-glucosamine) is a cationic polyelectrolyte with free amino groups distributed regularly in its molecular chain [3]. The properties that make chitosan commercially important are its biodegradability, biocompatibility in both plant and animal tissues, non-toxicity and non-allergenicity, as well as the ability to transform into gels, beads, fibres, colloids, films, flakes, powders and capsules [2, 4–6]. Additional exclusive characteristics of chitosan are its non-digestibility and bland taste that make it an excellent choice as a food additive [2].

By dissolving chitosan in organic acid aqueous solution a firm, transparent, colourless film can be made. The film shows good gas barrier and mechanical properties, but described characteristics vary among different reports, depending on the chitosan source, its characteristics, solvent used and the film production method [7]. Chitosan films were tested as carriers for low-molecular-weight active substances, such as organic acids, cinnamaldehyde or essential oils. It was proved that chitosan films enriched with oregano essential oil present an excellent system for controlled release of active compounds. Oregano essential oil also improved water vapour barrier properties of the film [8].

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Meat and meat products are highly susceptible to lipid oxidation and microbial spoilage, which lead to development of rancid or warmed-over flavour and other types of spoilage [9]. Meat products are commercially packed using vacuum or modified atmosphere conditions in high barrier plastic multilayer and combined films. Chitosan possesses antioxidant and antibacterial capacity [10], and may retard the lipid oxidation and inhibit the growth of spoilage bacteria in meat during storage. For this reason, it was tested for application in meat products, as an additive or active film with antimicrobial and antioxidative effect, as a natural alternative to plastic packaging.

Addition of chitosan reduced lipid oxidation, inhibited the growth of spoilage bacteria, resulted in better sensory attributes and had a good effect on the development of the red colour of minced beef during storage [11]. The results of adding chitosan to a "burger" model system indicated that high molecular weight chitosan had antioxidant activity, enhanced the red colour and reduced total viable counts of microorganisms [12]. It was shown that chitosan oligomer addition in emulsion-type sausage retarded lipid oxidation, without affecting overall acceptance of the sausage [13]. Research on storage stability of pork dipped in chitosan solution indicated that the dipping method was effective in extending the shelf life and preventing lipid oxidation of pork. The external redness of pork treated with chitosan remained unchanged during storage [14]. Chitosan added individually or in combination with nitrites showed significant effect in protecting fresh pork sausage from microbial growth and in reducing lipid oxidation [15]. Addition of a little amount of chitosan in Turkish sausage positively influenced its microbiological and sensory quality [16]. Combining good antimicrobial as well as antioxidative properties of chitosan and mint extract, the shelf life of pork cocktail salami was enhanced [17]. Combined chitosan-spice extracts coating showed a synergistic effect in antimicrobial as well as antioxidative activity and led to decreased moisture loss as well as better colour in chilled meat [18]. Cooked pork sausage wrapped with chitosan film or with chitosan – grean tea film showed lower thiobarbituric acid (TBA) values during twenty days of refrigerated storage [19]. Effectiveness of chitosan addition on storage stability of meat has been reported [9].

Addition of 0.02% of oregano essential oil to the bologna sausage reduced the levels of thiobarbituric acid reactive substances (TBARS) and increased the percentage of inhibition of the radical formation in DPPH (2,2-diphenyl-1-picrylhydrazyl) method [20]. Surface application of oregano essential oil reduced fungal contamination on the surface and led to a higher content of unsaturated fatty acids [21]. Direct addition of a natural oregano extract onto fresh beef steaks led to inhibition of metmyoglobin formation and showed significant inhibitory effect on lipid oxidation [22].

Two possible mechanisms of chitosan antioxidant action were presented. One is related to chelation of free iron that is released from hemoproteins of meat during heat processing. This in turn inhibits the catalytic activity of iron ions. The other mechanism involves the presence of a large number of ionic functional groups, which create strong polymer interactions thereby restricting the chain motion and resulting in good oxygen barrier properties [2]. Antioxidant action of the plant essential oil is related to presence of phenols due to the hydroxyl groups in their molecules. Oregano essential oil is rich in thymol and carvacrol, which are its main active compounds [8].

Chitosan film acts as a carrier matrix for essential oil. It may slow losses of volatile compounds of the oil and help the controlled release of active compounds during the extended time [8].

In this work, Petrovac sausage ("Petrovská klobása" label of geographical origin), a traditional dry fermented sausage, was produced following the original recipe, which includes only natural spices, without addition of synthetic additives. After production, chitosan film with oregano essential oil was applied on the sausage in an attempt to prolong the shelf life of the sausage without using plastic films for packaging. Sausage moisture content, degree of lipid oxidation and colour were measured after 150 days of storage. In addition, chitosan-laminated collagen casing was tested as a packaging material, regarding its mechanical and barrier characteristics.

MATERIALS AND METHODS

Reagents

Commercial highly viscous chitosan from crab shells was purchased from Sigma-Aldrich Chemical (St. Luis, Missouri, USA). Collagen casings were bought from a local manufacturer Cotex Viscofan (Novi Sad, Serbia). Oregano essential oil was purchased from manufacturer Aromara (Zagreb, Croatia). Glacial acetic acid and Tween 20 were obtained from Superlab (Belgrade, Serbia).

Film preparation

Chitosan film-forming solution was prepared by dissolving chitosan powder in acetic acid (1%

volume concentration) to reach chitosan mass per volume ratio of 4 kg·m^{-3}. Solution was stirred overnight on a magnetic stirrer in order to dissolve chitosan. Oregano essential oil (0.2% volume concentration) and the wetting agent Tween 20 (0.1% volume concentration) were added to the solution. The Petri dish was coated with 40 g of the film-forming solution to make chitosan film (CF). To make a laminated film (LF), collagen film surface was coated with tree layers of film-forming solution using a sponge brush. Both CF and LF were air-dried (temperature T = 23 °C ± 2 °C, relative humidity RH = 65% ± 2%) and conditioned for at least two days (T = 8 °C, RH = 65%) prior to analysis. Uncoated collagen film was used as a reference (C) [8].

Film characterization

Mechanical properties

Film thickness was measured using a micrometer with a sensitivity of 0.001 mm. Five thickness measurements were carried out on each film, from which an average was calculated.

Tensile strength (*TS*) and elongation at break (*EB*) of films were measured on the Instron Universal Testing Instrument Model No. 4301 (Instron Engineering, Canton, Massachusetts, USA) according to ASTM standard method D882-01 [23]. 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 100 mm·min^{-1}. *TS* and *EB* of the strips were measured in a static mode. *TS* (MPa) was calculated by dividing the given peak load by the cross-sectional area of the film. *EB* was calculated as the percent of change by dividing film elongation at the moment of rupture by initial gage length of the specimen (50 mm) and multiplying by 100 [23]. *TS* and *EB* measurements for each type of film were repeated five times, from which an average was calculated.

Water vapour barrier properties

Water vapour barrier properties of films were determined gravimetrically according to the ASTM E 96-95 desiccant method [24]. The method involves sealing a known open area of an impermeable container with the film being tested. Anhydrous silica gel was used to maintain a atmosphere of RH = 0% inside the cells. Distilled water was used to maintain RH = 100% outside the cells. Test cells were stored under a temperature of 23 °C ± 2 °C and weighed periodically until a constant rate of weight gain was reached. Obtained weight values were used for calculation of the amount of water vapour transferred through the film. The water vapour permeability (*WVP*) (g·m^{-2}d^{-1}kPa^{-1}) of the film was calculated (eq. 1).

$$WVP = \frac{\Delta w}{t \cdot a \cdot \Delta p} \quad (1)$$

where Δw (in grams) is the weight gain of test cells, t is the time between weighings (in this case, 24 h), a is the area of the exposed film (50 cm^2) and Δp is the partial water vapour pressure (in Pascals) difference across the two sides of the film. Under our conditions of determination, Δp was 1753.55 Pa.

Oxygen permeability

Oxygen permeability was measured using the method of Lyssy, according to DIN 53 380 [25] on the device Lyssy GPM-200 (Systech Instruments, Thame, United Kingdom) with a gas chromatograph GC-320 (Gasukuro Kogyo, Tokyo, Japan) and HP 3396 integrator (Hewlett-Packard, Palo Alto, California, USA). Measurements for each type of film were repeated five times, from which an average was calculated.

Scanning electron microscopy

Microstructure of film samples was studied by scanning electron microscopy (SEM), using the instrument JOEL 6 400 LV SEM (Japan Electron Optics, Tokyo, Japan). Samples were prepared using standard techniques, mounted on aluminium stubs and sputter-coated with gold (20 nm). Samples were observed using an accelerating voltage of 20 kV. For surface analysis, magnifications of 500× and 5 000× were used. For cross-sectional analysis, magnifications of 500× and 1 000× were used.

Production of Petrovac sausage

Sausage preparation

Sausages were made from lean pork meat and fat in a ratio of 80 : 20. Pork meat and fat were first grounded using a meat grinder PM-70/12 (Mainca, St. Louis, Missouri, USA) to a 10 mm particle size. Subsequemtly, spices were added in the following percentages: 2.50% red hot paprika powder, 1.80% salt, 0.20% crushed garlic, 0.20% caraway and 0.15% sugar. All ingredients were mixed for approximately 10 min, using a traditional technique. Mixture was then stuffed in collagen casings (55 mm diameter) using a vacuum filler (VF 50; Albert Handtmann Maschinenfabrik, Biberach, Germany) and left to drain for 12 h. No starter was added, thus fermentation was spontaneous. Drained sausages were taken to a smoking chamber were they were smoked in traditional manner, using a cool procedure for 12 days with pauses. Atmospheric conditions during smoking

were: T = 5–10 °C, RH = 75–85%, depending on outdoor atmospheric conditions. After smoking, sausages were processed in a drying room to reach 35.0% moisture content (48 days). During the drying phase, conditions were controlled at T = 8 –10 °C, RH = 90–75% [26–28].

A half of the produced sausages was coated with tree layers of a film-forming solution using a sponge brush (chitosan coated sausages). Every layer was left to dry overnight and then the next layer was applied. Other half of sausages was left uncoated (uncoated sausages), as a reference. After coating, sausages were stored in a chamber with a controlled temperature (15 °C) and relative humidity (75%) for five months. Analyses of moisture content, colour, lipid oxidation as well as sensory analysis of taste and smell were conducted before coating and on 150th day of storage (Fig. 1). All determinations were made in three samples from each batch (uncoated and chitosan coated sausages) in duplicate.

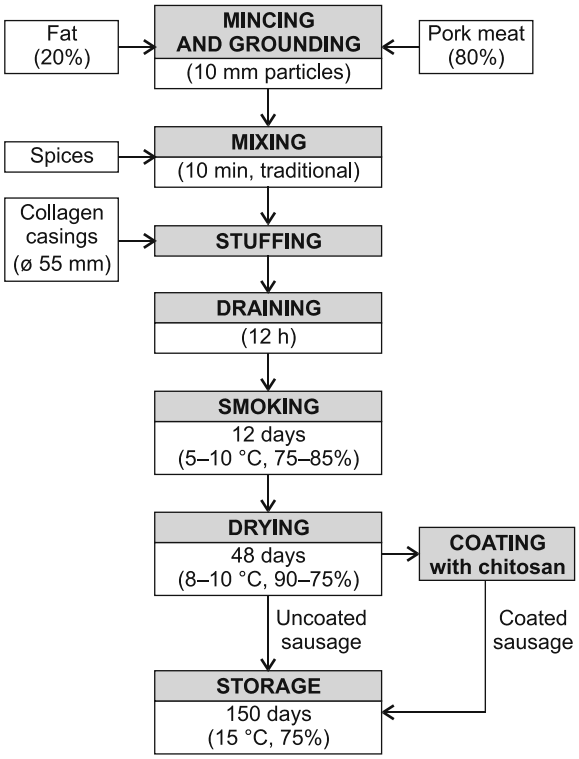

Fig. 1. Experiment scheme.

Uncoated sausage was used as a reference.

Sausage characterization

Moisture content

Samples were weighed (m_1), dried at 103 °C ± 2 °C to a constant weight and weighed (m_2) again. Moisture content (MC) was determined as the percentage of initial weight lost during drying and reported on a wet basis (eq. 2) [29].

$$MC = 100\frac{(m_1 - m_2)}{m_1} \quad (2)$$

Colour measurement

Colour measurements were taken immediately after cutting the samples to prevent colour degradation as a result of the action of light and oxygen, in accordance with the recommendations on colour determination of the American Meat Science Association [30]. Sample thickness was 3 cm.

Colour was studied in the CIE $L^*a^*b^*$ colour space and described by coordinates: lightness (L^*), redness (a^*) and yellowness (b^*). The CIE $L^*a^*b^*$ color coordinates were determined on the sausage core using Minolta Chromo Meter CR-400 with Light Protection Tube CR-A33b (Minolta, Osaka, Japan). Lighting D-65, standard observer angle of 2° and aperture of 8 mm were used. Before each set of measurements, the instrument was calibrated using a white ceramic tile (CR-A43).

Lipid oxidation analysis

TBA (2-thiobarbituric acid) measurements were conducted using the Rubio et al. method [31] with modification. Total volume of TBA was added to the sample and extraction was performed in an ultrasonic bath XUB 12 (Grant Instruments, Cambridge, United Kingdom), not in a mechanical homogenizer. Spectrophotometer Jenway 6300 (Jenway, Felsted, United Kingdom) was used. TBA values were expressed as milligrams of malonaldehyde per kilogram of sample.

Sensory evaluation of taste and smell

The casing was removed, the sausages were cut into slices of approximately 4 mm thickness and served at room temperature on white plastic dishes. Three slices were served from each batch. Water and unsalted toasts were provided to cleanse the palate between samples. Sensory evaluation of taste and smell was performed by a panel consisting of 7 trained members of different ages [32, 33]. Evaluation was performed according to quantitative descriptive analysis (QDA), using a scale from 0 to 5, with a sensitivity threshold of 0.25 points [34, 35]. Each mark means distinctive quality level, described as follows: 5 – extraordinary, typical, optimal quality; 4 – observable deviations or insignificant quality defects; 3 – drawbacks and defects of quality; 2 – distinct to very distinct drawbacks and defects of quality; 1 – fully changed, atypical properties, product un-

acceptable; 0 – visible mechanical or microbiological contamination, atypical product.

Statistical analysis

Statistical analysis was carried out using Origin pro 8.0 (OriginLab, Northampton, Massachusetts, USA). All data were presented as mean values with standard deviation (mean ± SD). Pair sample *t*-test was applied to compare the means of sausage properties and one-way ANOVA test was applied to compare the means of film properties. Differences were accepted as significant when $p \leq 0.01$.

RESULTS AND DISCUSSION

Film characterization

Thickness was similar for the laminated film and collagen film, 78 μm ± 13 μm and 76 μm ± 13 μm, respectively (Tab. 1). Thickness of chitosan film was 40 μm ± 8 μm. Collagen film strength (60.63 MPa ± 17.16 MPa) decreased after lamination (45.69 MPa ± 14.93 MPa) to a strength similar to chitosan film (46.72 MPa ± 7.97 MPa). Strength of laminated film is comparable to plastic films, such as low-density polyethylene (23.60 MPa) and high-density polyethylene (47.40 MPa) [35]. *EB* values of laminated film were poor compared to aforecited plastic films, which have *EB* values above 200% [36]. Laminated film had *EB* = 6.0% ± 2.9%, while both collagen and chitosan films had *EB* values under 10%. Mechanical characteristics of the films were in the range of values presented for a chitosan film with added 1% oregano essential oil: *TS* about 50 MPa and *EB* about 10% [8]. *TS* of 33.82 MPa and *EB* of 2.30% were presented by PRANOTO et al. for a chitosan film with 200 μl garlic essential oil per gram of chitosan [37]. These values mean that films prepared in this study were relatively strong but brittle.

According to sausage manufacturing process, high water permeability is important for collagen film in order to enable drying. Once sausage manufacturing process is over, further moisture loss should be stopped or slowed down. Laminated film prepared in our study had water vapour permeability of 212.48 $g \cdot m^{-2} d^{-1} kPa^{-1}$ ± 27.96 $g \cdot m^{-2} d^{-1} kPa^{-1}$, which was not significantly lower than for collagen film (217.28 $g \cdot m^{-2} d^{-1} kPa^{-1}$ ± 4.34 $g \cdot m^{-2} d^{-1} kPa^{-1}$) and chitosan film (213.25 $g \cdot m^{-2} d^{-1} kPa^{-1}$ ± 5.66 $g \cdot m^{-2} d^{-1} kPa^{-1}$; Tab. 1). Compared to 90 μm polyethylene, which showed water vapour permeability of 0.03 $g \cdot m^{-2} d^{-1} kPa^{-1}$ [38], all tested films showed high values of this parameter. Comparable results were presented by KANDASAMY for water vapour permeability of four different types of chitosan films (four different methods of chitosan production): 156.34 $g \cdot m^{-2} d^{-1} kPa^{-1}$, 138.24 $g \cdot m^{-2} d^{-1} kPa^{-1}$, 172,80 $g \cdot m^{-2} d^{-1} kPa^{-1}$, 189.42 $g \cdot m^{-2} d^{-1} kPa^{-1}$ [39]. Similar results were presented for the chitosan film, 204.63 $g \cdot m^{-2} d^{-1} kPa^{-1}$ and chitosan film with added 0.4% cinnamon essential oil, 119.20 $g \cdot m^{-2} d^{-1} kPa^{-1}$ [40]. These values mean that laminated film prepared in this study should be further improved to optimise its water barrier properties. Collagen film is permeable for gasses and this property is very important for its application in sausage manufacturing, especially for the smoking phase. When sausage is manufactured, further contact with air oxygen can leads to undesirable changes, such as discoloration and lipid oxidation. Chitosan film is a very good barrier for air oxygen. After lamination, collagen film oxygen permeability lowered from 22 799.3 $ml \cdot m^{-2} d^{-1} MPa^{-1}$ ± 7 924.6 $ml \cdot m^{-2} d^{-1} MPa^{-1}$ to 48.0 $ml \cdot m^{-2} d^{-1} MPa^{-1}$ ± 45.9 $ml \cdot m^{-2} d^{-1} MPa^{-1}$. These values indicate lipid oxidation and discoloration of sausages may be retarded. In the literature, chitosan film was reported to have oxygen permeability of 454.55 $ml \cdot m^{-2} d^{-1} MPa^{-1}$ [41]. For comparison, 90 μm polyethylene had oxygen permeability of 24 392.0 $ml \cdot m^{-2} d^{-1} MPa^{-1}$ [38].

The layer of chitosan continuously covered the collagen base surface, as it could be seen by scanning electron microscopy of the laminated film surface (Fig. 2). Unevenness of the collagen base could still be seen after lamination. On a 500×

Tab. 1. Film characteristics.

Film	Thickness [μm]	Tensile strength [MPa]	Elongation at break [%]	Water vapour permeability [$g \cdot m^{-2} d^{-1} kPa^{-1}$]	Oxygen permeability [$ml \cdot m^{-2} d^{-1} MPa^{-1}$]
Collagen	76 ± 13^{A}	60.63 ± 17.16^{ns}	9.2 ± 4.0^{ns}	217.28 ± 4.34^{ns}	22799.3 ± 7924.6^{A}
Chitosan	40 ± 8^{B}	46.72 ± 7.97^{ns}	6.8 ± 4.1^{ns}	213.25 ± 5.66^{ns}	301.4 ± 127.2^{B}
Chitosan laminated collagen	78 ± 13^{A}	45.69 ± 14.93^{ns}	6.0 ± 2.9^{ns}	212.48 ± 27.96^{ns}	48.0 ± 45.9^{B}

A, B – letters mark significantly different means with 99% probability ($p < 0.01$); ns – letters mark means that are not significantly different ($p > 0.01$).

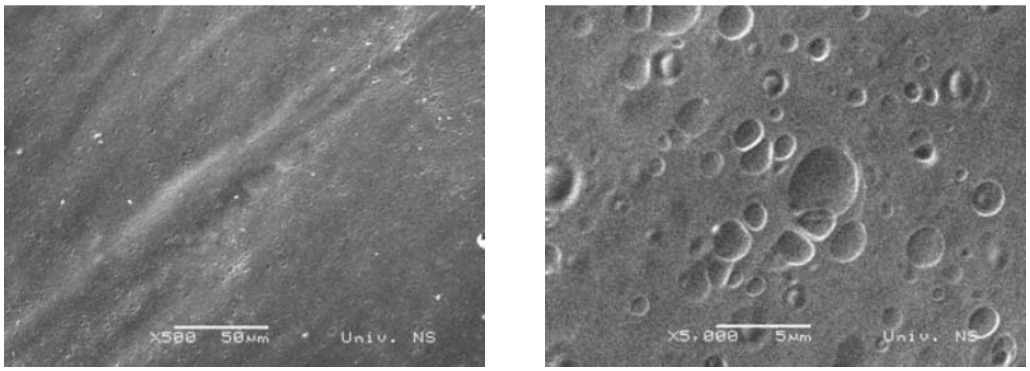

Fig. 2. Scanning electron microscopy of laminated film surface: 500× and 5 000× magnified.

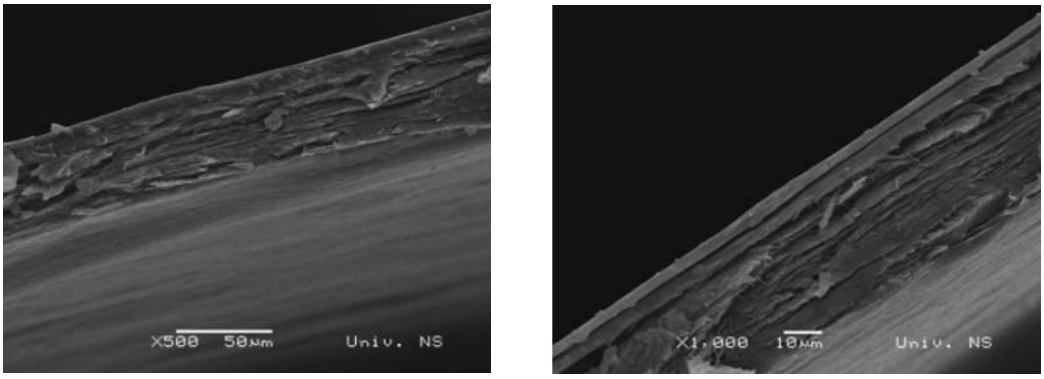

Fig. 3. Scanning electron microscopy of the cross sectional surface of laminated film: 500× and 1 000× magnified.

magnified SEM image, little pores from air bubbles could be seen, as well as granules of undissolved chitosan. During drying, air bubbles moved from the mass of the film-forming solution to the surface, leaving traces on the film. At 5 000× magnification, these traces could be clearly seen, being smaller than 5 μm in diameter.

On SEM images of laminated film cross-section, multilayered structure could be seen (Fig. 3). At 500× magnification, the chitosan top layer could be noticed to adhere completely to the chitosan base. Further magnification (1 000×) showed that chitosan top layer consisted also of two layers, and that adhesion was not complete through the entire surface.

Sausage characterization

Moisture content is one of the prescribed quality parameters of the fermented dry sausages, according to the Serbian Regulation of quality and other requirements for meat products [27]. This type of sausage must meet the requirement that the maximum moisture content does not exceed 35%. It can be concluded from the results presented in the Tab. 2 that the examined samples satisfied the requirements of the Regulations. After reaching 35% or less moisture content, further moisture loss is undesirable because it affects sensory quality and sausage appears too dry, tough and wrinkled. During storage, moisture content decreased both in chitosan coated and uncoated sausages. This decrease was slightly less pronounced for chitosan coated sausage. Coating sausages with chitosan layer slowed down the moisture loss, but moisture loss was still significant after 150 days of storage. These results mean that laminated film should be further optimized regarding its moisture barrier properties in order to prolong the shelf life of sausages (Tab. 2).

Malonylaldehyde, the main degradation product of lipid peroxides, was used as a marker for the determination of lipid peroxidation degree. The results of TBARS test, expressed as malonylaldehyde content in milligrams per kilogram, are presented in Tab. 2.

TBA method is a relative method used only for treatment comparison as TBA values vary considerably in studies of different authors. TBA

Tab. 2. Sausage moisture content, TBARS and CIE colour parameters.

Parameters		Sample	Storage	
			0 days	150 days
Moisture content [%]		Uncoated sausage	32.3 ± 0.1	18.4 ± 0.1^{A}
		Chitosan coated sausage		19.3 ± 0.1^{B}
TBARS [mg·kg^{-1}]		Uncoated sausage	5.47 ± 0.15	29.23 ± 0.00^{A}
		Chitosan coated sausage		9.64 ± 0.08^{B}
Colour	*L**	Uncoated sausage	39.12 ± 6.99	27.69 ± 5.20^{ns}
		Chitosan coated sausage		26.50 ± 2.61^{ns}
	*a**	Uncoated sausage	25.90 ± 6.15	19.86 ± 3.39^{ns}
		Chitosan coated sausage		19.70 ± 2.61^{ns}
	*b**	Uncoated sausage	23.18 ± 4.02	16.90 ± 6.03^{ns}
		Chitosan coated sausage		15.51 ± 3.38^{ns}
Sensory evaluation		Uncoated sausage	4.15 ± 0.19	2.95 ± 0.31^{A}
		Chitosan coated sausage		3.95 ± 0.10^{B}

TBARS values are expressed as malonylaldehyde content.
A, B – letters mark significantly different means with 99% probability ($p < 0.01$); ns – letters mark means that are not significantly different ($p > 0.01$).

values from this paper were in accordance with results obtained by some researchers [31, 42], but much higher than results of others for the same product type [43]. Uncoated sausages after 150 days of storage showed very high TBA values. It can be seen in Tab. 2 that chitosan coated sausages had significantly lower TBA values than uncoated. This could be due to antioxidant activity of chitosan [9–19], antioxidant activity of oregano [20–22] or synergistic effect of these two. This is a valuable result, which can be used in further studies on Petrovská klobása shelf life prolongation using chitosan-based coatings.

Compared to day 0, both uncoated and chitosan coated sausages were scored with lower scores for taste and smell after 150 days of storage. With uncoated sausages, panelists noticed drawbacks and defects of taste and smell in the form of slight rancidity. For chitosan coated sausages, a slight oregano aroma was detected. No other changes in taste or smell were detected but, because of an atypical aroma, chitosan coated sausages was scored lower after storage than the starting sausage. These results correspond to TBA test results. Rancidity was avoided in chitosan coated sausages but, in order to avoid changes in the characteristic taste and smell, it might be useful to replace oregano essential oil with essential oil originating from spices that are included in the recipe for "Petrovská klobása".

The instrumentally determined colour of the sausages showed similar L^* values and significantly higher a^* and b^* parameter values than the results other researches obtained for sausages of a similar types [31, 44]. It should be noted that red spice paprika and components of smoke in the surface casings probably had the greatest influence on the share of the red colour in sausages [44]. The loss of moisture from the product increased the content of myoglobin and, on the other hand, the dehydrated muscle tissue absorbed a greater amount of light rays, which resulted in a darker colour of the products reflected by a decrease in L^* values [44]. Decrease of red colour (a^*) of the surface of cut sausages was also caused by the contained red spice paprika, components of which started to oxidize for a number of reasons during drying and ripening of the sausage. Also, coloured smoke fractions (neutral and phenol compounds) are known to affect the formation of specific dark red-brown colour of the cut surface of the examined sausages (Petrovská klobása) [45] and therefore affected the share of the red colour (a^*). Increased content of NaCl is known to reduce the amount of yellow colour [44], which is in agreement with results obtained in this study.

There was no difference in the colour of chitosan-coated sausages, compared to uncoated sausage.

CONCLUSION

Lamination of "Petrovská klobása" with chitosan layer had positive effect in slowing down the deterioration of the sausage. Lipid oxidation

was slowed down, probably due to chitosan and oregano antioxidant activities and good air oxygen barrier properties. In sensory evaluation of taste and smell, chitosan-laminated sausage was favoured, considering taste and smell, after five months of storage. Moisture loss was slowed down by coating the sausage, although water vapour barrier properties of laminated film were not significantly different compared to collagen casings. These results are encouraging for further work on optimization of chitosan based coating (water vapour barrier, chitosan as a natural additive carrier) for dry fermented sausage shelf life prolongation, without using plastic films and special packaging conditions.

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