www.foodandfeed.fins.uns.ac.rs
FOOD AND FEED RESEARCH
Journal of the Institute of Food Technology – FINS
University of Novi Sad
ISSN:
2217-5369 (print),
2217-5660 (online)
University of Novi Sad

UDK 664.848:664.151.2]:577.11+547.962.7:621.796

Original research paper

DOI: 10.5937/ffr0-35821

INFLUENCE OF BIOPOLYMER COATINGS ON THE STORAGE STABILITY OF OSMOTICALLY DEHYDRATED MUSHROOMS

Danijela Z. Šuput[*1], Vladimir S. Filipović[1], Biljana Lj. Lončar[1], Milica R. Nićetin[1], Violeta M. Knežević[1], Jasmina M. Lazarević[2], Dragana V. Plavšić[2], Senka Z. Popović[1], Nevena M. Hromiš[1]

[1]University of Novi Sad, Faculty of Technology Novi Sad, 21000 Novi Sad, Bulevar cara Lazara 1, Serbia
[2]University of Novi Sad, Institute of Food Technology, 21000 Novi Sad, Bulevar cara Lazara 1, Serbia

Abstract: The main aim of this research was to apply biopolymer coatings on osmotically dehydrated mushrooms and monitor their quality during storage. Mushrooms were osmotically dehydrated in sugar beet molasses (80% dry matter) under optimized conditions (45 °C for 5 hours), as previously reported elsewhere. Two different biopolymers were chosen: chitosan, a polysaccharide polymer, and zein, a protein polymer. A non-treated mushroom sample was chosen as a control sample. The mushroom samples were analysed for sugar and protein content, as well as water loss and microbiological profile. An increase in sugar content was the most noticeable in the osmotically dehydrated mushrooms compared to the control sample due to the use of molasses as a hypertonic solution. The contribution of used biopolymer coatings to the sugar and protein content of the coated and osmotically treated mushrooms was negligible. Chitosan coating contributed to better storage stability of treated mushrooms by lowering the moisture loss and microbial count. For this reason, chitosan treated sample was chosen for further examination related to the evaluation of its baking potential as a filling in a traditional stuffed pie-like layered bakery product-burek. Burek was stuffed with fresh mushrooms, osmotically treated mushrooms or osmotically treated mushrooms coated with chitosan. The sensorial assessment proved that control burek and burek samples with osmotically dehydrated mushrooms coated with chitosan were the most preferred groups based on odour and overall impression.

Key words: *molasses, mushrooms, zein, chitosan, storage stability, burek, sensory properties*

INTRODUCTION

Mushrooms are a valuable horticultural crop because of their nutritional value, as well as medicinal qualities such as anti-inflammatory, cardiovascular, anticancer, antiviral, antibacterial, etc. (Karimirad, Behnamian & Dezhsetan, 2018; Wang et al., 2021). Mushrooms are high in protein and carbohydrate, low in fat, contain nine essential amino acids and are a rich source of vitamins B and D (Rathore, Prasad & Sharma, 2017; Cardwell, Bornman, James & Black, 2018; Samsudin & Abdullah, 2019). *Agaricus bisporus* mushroom is a popular food item worldwide, accounting for 30% of total mushroom production. Because there is no protective cuticle layer on the skin of *Agaricus bisporus*, it is prone to physical and microbiological harm

Corresponding author: Phone: +381214853702 Fax: +38121450413
E-mail address: suput.danijela@gmail.com

(Zhang, Pu & Sun, 2018). Due to high metabolic activity, high respiratory rate, high moisture content (85%–95%), enzymes activeties and dehydration (Kumar, Singh & Singh, 2013; Zhang et al., 2018), mushrooms are perishable foods with a limited shelf life and tend to lose quality immediately after harvest (Jiang, 2013). All of the listed reactions promote microbial attack and enzymatic browning, resulting in fast senescence (Wrona, Bentayeb & Nerín, 2015). The shelf-life of *A. bisporus* mushrooms is about 1–3 days when stored at room temperature that could be prolonged to 5–7 days when stored at 0–2 °C (Diamanto-poulou & Philippoussis, 2015; Xu, Tian, Ma, Liu & Zhang, 2016).

As a result, it is necessary to use proper presservation techniques to extend the postharvest storage period and maintain mushroom quality (Gholami, Ahmadi & Farris, 2017). Osmotic dehydration is a solid-liquid contact operation involving the immersion of food products in hypertonic solutions (Pacheco-Angulo, Herman-Lara, García-Alvarado & Ruiz-López, 2016). This procedure results in partial dewatering of food material and simultaneous solute uptake from the solution into the product (da Silva Junior, da Silva, de Farias Aires, Aires & de Castro, 2017). Osmotic dehydration is usually a pretreatment procedure that is frequently followed by additional drying methods such as air drying, deep fat frying, freeze-drying, etc., to produce a higher-stability final product with reduced water content (Khan, 2012; Phisut, 2012). These food products are defined as minimally processed, high-quality products with prolonged shelf life. They are becoming more popular because their qualities are comparable to those of fresh food products, with the additional advantage of more extended shelf-life (Qiu, Zhang, Tang, Adhikari & Cao, 2019). However, the microbiological load of osmo-dehydrated food products is a major problem for their improvement, since it may affect the shelf life, particularly that of cut fruits (Castelló, Igual, Fito & Chiralt, 2009). Biopolymer coating application is a viable option for lowering a microbial load and postharvest losses, thereby providing added value to food products.

Biopolymers have attracted a lot of interest in the packaging industry because of their availability, ease of processability and biodegradability, making them viable alternatives to fossil-based plastics (Rangaraj, Rambabu, Banat & Mittal, 2021). Biopolymer coatings have been regarded as an effective alternative method to extend the commercial shelf life of food products because the major ingredients of edible coatings, such as proteins, lipids, and polysaccharides, are mainly made from various agricultural products and food processing wastes and by-products (Šuput, Lazić, Popović & Hromiš, 2015; Vieira et al., 2016; Maqbool, Ali, Alderson, Zahid & Siddiqui, 2011). In addition, green technologies are gaining popularity due to their environmental benefits and safety for human health (Wang et al., 2021). Biopolymer coating application represents powerful emerging technology that can prevent the food products from mechanical damage, lower lipid oxidative changes, maintain microbiological control, reduce volatiles loss, preserve sensory properties, etc. (Alemán et al., 2016; Qiu et al., 2019). The application of alginate edible coating maintained mushrooms' firmness, delayed discolouration and inhibited the loss of soluble solids concentration, total sugars and ascorbic acid of mushrooms (Jiang, 2013). Chitosan-glucose coating-maintained tissue firmness, reduced microbial counts and inhibited the increase of respiration rate in mushrooms within 16 days of storage at 4 °C (Jiang, Feng & Li, 2012). Mohebbi, Ansarifar, Hasanpour and Amiryousefi (2012) proved that aloe vera and gum tragacanth edible coatings minimized mushroom weight loss, colour changes and texture softening during 13 days of cold storage at 4 °C.

Chitosan, a linear polysaccharide, is considered an interesting material in food packaging because of its non-toxic, bacteriostatic, fungistatic, anti-cancer properties, good biodegradability and biocompatibility characteristics, as well as naturally occurring and quick gelling ability (Karimirad et al., 2018). Zein is a class of alcohol-soluble prolamin storage protein in corn. Pure zein is clear, odourless, tasteless, hard, water-insoluble, and edible, which was approved as generally recognized as safe (GRAS). In addition, corn zein is a relatively hydrophobic and thermoplastic material with excellent film-forming properties and can be used as a good renewable and biodegradable material for food packaging applications (Zhang et al., 2015). Chitosan and zein biopolymer coatings were prepared and applied on the osmotically dehydrated mushrooms, whose quality was monitored during storage.

In the light of these considerations, the present study was undertaken to complete two tasks. The first task was to investigate the effect of biopolymer coatings on the quality and storage stability of osmotically dehydrated mushrooms in sugar beet molasses. Sugar beet molasses was chosen for its high content of solids and rich nutritional composition (Šarić et al., 2016). A previous study investigated by Šuput et al. (2020) revealed that optimal processing parameters for dehydration in sugar beet molasses were: process time of 5 hours, process temperature of 45 °C and molasses concentration of 80%. Chitosan and zein were chosen as coating bases.

The second task was to evaluate the baking performance of treated mushrooms as a filling in a popular baked food, burek (börek) – a traditionnal pie-like dish stuffed with savoury fillings. Three burek types were prepared: 1) with fresh mushrooms, 2) with osmotically dehydrated mushrooms, and 3) with osmotically dehydrated mushrooms coated with a biopolymer with the best-preserving properties. The prepared burek samples were assessed for proximate analysis and sensory acceptance.

MATERIALS AND METHODS

Materials

Commercial chitosan powder from crab shells, middle viscous, was purchased from Sigma-Aldrich Chemical Co. (USA), while purified zein, was purchased from Acros Organics (Belgium). Acetic acid (glacial 99.5%) was provided by Macron (USA) and ethanol (96%) by Reahem (Serbia). Polyethylene glycol PEG 400 was procured from Alfa Aesar (Germany). Sugar beet molasses (pH 8.2 and 80% dry matter) was procured from the sugar factory Crvenka (Serbia).

Experimental design

The experiment involved 4 groups of treatments/samples:

K – control sample: non-treated mushrooms;

OD – mushrooms osmotically treated in molasses;

OD+C – osmotically treated mushrooms coated with chitosan film;

OD+Z – osmotically treated mushrooms coated with zein film.

After the treatments, the mushrooms were analysed for quality changes during a 10-day storage period. The mushroom samples were packed in polypropylene bags in an air atmosphere and stored in the refrigerator at 4 °C. The samples were examined on the 1st, 2nd, 4th, 7th and 10th day of storage.

The treated mushrooms with the best storage stability were chosen for further examination related to their baking potential as a filling in a traditional stuffed pie-like bakery product-burek.

Osmotic dehydration

Fresh mushrooms *(Agaricus bisporus)* were bought at a local greengrocery and processed the same day. Osmotic dehydration was performed by the method previously described by Šuput et al. (2020): Mushrooms were cleaned, dried with a cloth and sliced into 5 mm-slices before being immersed in sugar beet molasses diluted to 80% concentration in a 1:5 weight ratio (mushrooms:molasses). Osmotic dehydration was carried out for 5 hours in a constant-temperature chamber (KMF 115 l, Binder, Germany) at 45 °C under atmospheric pressure. Every 15 min, the osmotic solutions with mushrooms were stirred to provide better homogenization, considering the amount of diffused water from the samples. After removal from the solution, the mushroom samples were quickly cleaned with tap water and absorbed from excess water.

Biopolymer coating preparation and application

Chitosan film-forming solution was prepared by dissolving chitosan powder in acetic acid (1% volume concentration) to reach a chitosan mass per volume ratio of 10 kg/m^3. The solution was left stirring overnight on a magnetic stirrer to dissolve chitosan.

Zein was dissolved (10% w/v in 85% ethanol) and 0.5 g PEG 400/g zein was added as a plasticizer and stirred until complete dissolution. The film-forming solution was heated in a water bath at 80 °C for cca 5 minutes.

Mushroom samples were separately immersed in both biopolymer solutions for 1 min and left to drain for 3 min. The dipping procedure was repeated 3 times. The control osmotic dehydrated sample was not coated. After immersion, the samples were left in the strainer for half an hour to drain off the excess film solution and then packaged. The mushroom samples were packed in polypropylene bags in an air atmos-

phere and stored in the refrigerator. The samples were examined on the 1st, 2nd, 4th, 7th and 10th day of storage.

Methods for mushroom quality determination

Weight loss

Weight loss was calculated as the percentage of weight loss with respect to the initial weight. The weight of five mushrooms from each package was determined at the initial time and sampling times. Weight loss was determined gravimetrically using the following formula:

$WL\ (\%) = (m_1 - m_2)/m_1 \times 100\%,$

where m_1 is the initial mass of mushrooms before storage (g), and m_2 is the mass of mushrooms at each sampling time (g). The results were expressed as the percentage loss of initial weight.

Protein content

The protein content was determined according to the method SRPS ISO 937:1992.

Microbiological analysis

The presence of *Enterobacteriaceae* was determined according to SRPS ISO 21528-2:2017. The determination of *Escherichia coli*, presence was performed on the basis of the standard SRPS ISO16649-2:2008. The determination of *Salmonella* spp. was made according to the standard SRPS EN ISO 6579:2017. The presence of *Listeria monocytogenes* was determined according to the standard SRPS EN ISO 11290-2:2017. The number of yeasts and molds was determined according to the standard SRPS ISO 21527-2:2011.

Burek preparation

The basic dough was prepared according to the formula 700 g of dough with 300g of filling. Dough was prepared with extra white, wheat flour (type 400, ash content max. 0.45% dry basis, coarse granulation) with the addition of 1.5% salt, 1% sugar and 3% sunflower oil for kneading plus oil to brush on the pastry and 40% of water (percentages on dough basis), at a temperature of 15 °C. Dough was mixed in a high-speed spiral mixer (Prominent & Leading Manufacturer, Muzaffarnagar) for 5 min. The dough was divided into four parts and formed into balls. Afterwards, the dough was subjected to relaxation for 20 min and then stretched out all over the table by hand. During stretching, the thickness of the dough was progressively reduced to 3 mm.

Burek filling was prepared by 15-min stewing at 90 ^{0}C of chopped onion and treated mushrooms. The filling was seasoned with cca. 12 g of a mixture of salt and pepper. The dough was stretched, filled with 300 g mushroom filling and shaped. The burek was then baked in a bakery oven at 200-210 °C, 15-20 minutes and cooled down for 20 minutes at room temperature. The samples were analyzed fresh, immediately after baking. The control burek sample was prepared with non-treated mushrooms.

Methods for burek quality determination

Nutritional composition and energy value

Fat content (according to Weibull and Stoldt) was determined according to method No. 2.4. of the National rulebook of standard methods (Pravilnik, 1988. Protein content was determined according to the Kjeldahl method (method No. 2.3.); NaCl content was determined according to the method No. 2.6. and total sugar content (according to Luff-Schoorl) was determined in accordance with the method No 2.9 of the aforementioned rulebook. Energy value was calculated using the conversion factors (Pravilnik, 2018).

Sensory evaluation

Sensory evaluation of burek filled with mushrooms was carried out by a semi-trained panel of 10 assessors with extensive experience and training in sensory methodology. The sensory test was performed in tasting booths with properly controlled environment conditions (ISO, 2007).

Samples were evaluated immediately after baking. Drinking water was provided for palate cleansing between each sample. Four 2-hr sessions were devoted to techniques and practice in attributes generation. The attributes were generated and chosen based on their uniqueness and objectivity.

Descriptive terms developed in the assessment of sensory quality and intensity of each property were developed using terms from the lexicon of SRPS ISO 5492 (2000) - Sensory analysis-Vocabulary. The sensory list attributes considered and the descriptor definitions according to Hozová, Kukurová, Turicová and Dodok (2002)

Table 1.
Descriptors for sensory evaluation

Descriptor (attribute)		Definition (characteristics)
Product appearance (shape and volume)		
	5	regular, well-formed
	4	regular, slightly formed
	3	slightly regular, slightly formed
	2	irregular
	1	deformed
Top surface appearance		
	5	golden-brown, fragile, smooth
	4	darker or lighter, less- fragile, smooth
	3	darker or lighter, medium-fragile, smooth
	2	darker or lighter, medium-fragile, fine-cracked
	1	very dark or light, non-fragile, more cracked
Internal appearance-crumb structure		
	5	soft, fluffy, puffy layers
	4	soft, fluffy, slightly puffy layers
	3	soft, fluffy, undesirable merged layers
	2	slightly thick merged layers
	1	very thick merged layers
Odour		
	5	characteristic, harmonizing with mushrooms stuffing
	4	less harmonizing with mushrooms stuffing
	3	slightly harmonizing with mushrooms stuffing
	2	odd intense caramel-like from molasses
	1	strongly disagreeable, odd caramel-like from molasses
Taste		
	5	delicious, very agreeable pie-like, harmonizing with mushrooms stuffing
	4	delicious, lightly harmonizing with mushrooms stuffing
	3	strong expressive, sweet taste of molasses
	2	slightly disagreeable, salty, intense, caramel–like from molasses
	1	strongly disagreeable, odd
Overall impression		
	5	very agreeable, quite satisfactory
	4	suitable, insignificant deviations
	3	visible deviations
	2	large deviations
	1	unacceptable

with some modifications are presented in Table 1. Using a 5-point hedonic scale, according to which the maximum value of 5 points corresponds to the highest degree of the evaluated product's quality whereas the lowest degree of the evaluation expressed by 1 point demonstrated its fundamental qualitative deficiencies, the quality of burek was evaluated according to the QDA method (Hozová et al., 2002). The evaluated parameters were the following: shape, surface appearance, internal appearance, odor, taste and overall impression.

Statistical analysis

The results were analyzed by one-way analysis of variance (ANOVA) using Statistica Ver. 12.0., StatSoft Inc., (Tulsa, OK, USA) to determine significant differences between samples. All analysis were performed in 3 replicants. Significance testing was performed by Tukey's test, and the differences were considered statistically significant when the p-value was < 0.05.

RESULTS AND DISCUSSION

Influence of OD treatments and coating application on mushroom quality

Weight loss

Due to the lack of a protective epidermal structure, weight loss, the key predictor of mushroom storage quality, is mainly related to respiration and moisture evaporation via the

surface (Huang, Qian, Jiang & Zheng, 2019; Louis et al., 2021). This parameter is critical, as fruit and vegetables with a weight loss of greater than 4–6% (of their initial weight) showed evident withering or shrivelling symptoms (Bico, Raposo, Morais & Morais, 2009). Fig. 1 depicts the weight loss differences between the treated and non-treated mushrooms during a 10-day storage period. Due to continuous moisture loss over time, weight loss increased with storage time for all tested samples. After ten days of storage, the OD+C samples showed the lowest weight loss (3.3%), followed by OD+Z (3.8%), OD (6%), and finally, the control sample (11.4%).

Protein and sugar content

The protein and sugar contents of treated mushrooms were determined to test whether biopolymer coatings affect their content since chitosan is a polysaccharide and zein is a protein. Changes in protein and sugar content during the storage period are shown in Table 2. A statistically significant difference in protein and sugar content was observed between the control sample (K) and the rest of the other samples (OD, OD+C, OD+Z). Storage period (time) did not significantly influence protein and sugar content.

According to the obtained results, lower protein content was observed in osmotically dehydrated samples compared to the control non-dehydrated sample, which is in agreement with previous findings (Šuput et al., 2020). Sugar solid gain occurred during dehydration in sugar beet molasses along with protein leakage into the molasses solution, which, as a counter-current flow, resulted in lower protein values. Within the group of dehydrated samples, protein content during storage ranged from 16.47-18.60% d.m. (d.m.-dry matter). Discretely higher protein content values were in the sample of osmotically dehydrated mushrooms coated with zein, which is a consequence of the very protein nature of this biopolymer.

Sugar content was the lowest in the control sample. The highest increase in sugar content was observed between the control sample and all other osmotically dehydrated mushroom samples due to molasses impact, which is in agreement with previous findings (Šuput et al., 2020).

Microbiological analysis

High water content and a neutral pH in mushrooms provide an ideal medium for microbial growth (Zhang et al., 2018). The results of microbiological assays showed that *E. coli*, *Salmonella spp*. and *Listeria monocytogenes* were not detected in any of the examined sample groups. Table 3 presents results related to the total counts of yeasts and moulds as well as Enterobacteriaceae.

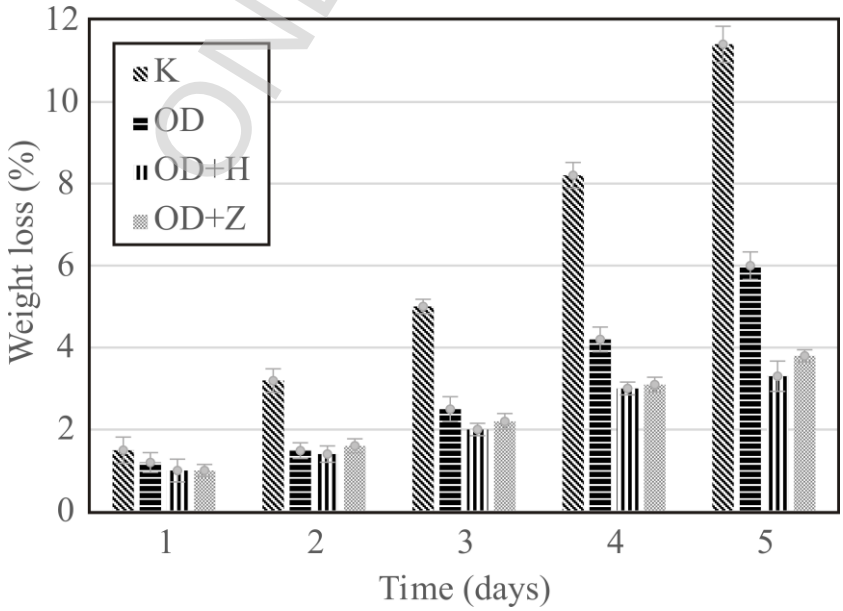

Figure 1. Weight loss for control mushroom samples (K), osmotically dehydrated mushroom samples (OD), osmotically dehydrated mushroom samples coated with zein (OD+Z) and osmotically dehydrated mushroom samples coated with chitosan (OD+C)

Table 2.
Protein and sugar content in control mushroom samples (K), osmotically dehydrated mushroom samples (OD), osmotically dehydrated mushroom samples coated with chitosan (OD+C) and osmotically dehydrated mushroom samples coated with zein (OD+Z)

Storage period (days)	Protein content ($\%_{d.m.}$)				Sugar content ($\%_{d.m.}$)			
	K	OD	OD+C	OD+Z	K	OD	OD+C	OD+Z
1	23.21± 1.10 [b]	16.99± 0.84 [a]	16.42± 0.80 [a]	18.60± 0.95 [a]	27.35± 1.35 [a]	37.82± 1.90 [b]	38.00± 1.87 [b]	37.58± 1.89 [b]
2	23.01± 1.13 [b]	16.72± 0.86 [a]	16.47± 0.83 [a]	17.21± 0.87 [a]	27.47± 1.39 [a]	37.85± 1.87 [b]	37.96± 1.90 [b]	37.81± 1.85 [b]
4	23.32± 1.16 [b]	17.08± 0.82 [a]	16.67± 0.83 [a]	17.70± 0.85 [a]	27.21± 1.33 [a]	37.95± 1.88 [b]	38.11± 1.88 [b]	37.87± 1.86 [b]
7	23.34± 1.14 [b]	17.19± 0.87 [a]	16.89± 0.85 [a]	17.23± 0.82 [a]	27.06± 1.35 [a]	37.90± 1.86 [b]	38.16± 1.91 [b]	37.92± 1.90 [b]
10	23.51± 1.13 [b]	17.20± 0.81 [a]	16.88± 0.83 [a]	17.35± 0.84 [a]	27.49± 1.38 [a]	37.94± 1.89 [b]	38.21± 1.89 [b]	38.00± 1.89 [b]

[a-b] Different letters in superscript for data set regarding each chemical response (protein and sugar content) indicate statistically significant difference among means, at level of significance of $p<0.05$ (based on post-hoc Tukey (HSD) test)

The control sample had the highest value of cfu/g for yeasts and moulds and ranged between $4 \cdot 10^3$ and $4.8 \cdot 10^{3,}$ respectively, during the entire storage period. The control sample only in the last sampling (10^{th} day) had a statistically significant increase in yeast and moulds compared to other samples, i.e., storage period (time) had a statistically significant influence on yeast and moulds increase in the last sampling. In OD and OD+Z samples, there was a statistically significant increase in yeasts and moulds on the 4^{th} day of storage. The sample OD+C stands out concerning a statistically significant decrease in the number of yeasts and moulds after the 4^{th} day, which was below the detection limit. This is in agreement with previous studies, that also proved that chitosan-based coatings could prolong the postharvest life of some fruits and vegetables: strawberries (Perdones, Sánchez-González, Chiralt & Vargas, 2012), bananas (Maqbool et al., 2011), cherry (Xin, Chen, Lai & Yang, 2017), plum (Kumar, Sethi, Sharma, Srivastav & Varghese, 2017), kiwifruit (Kaya, Česonienė, Daubaras, Leskauskaitė & Zabulionė, 2016). Similar results were obtained when examining Enterobacteriaceae. Enterobacteriaceae of the control sample continuously increased during time achieving the maximum value ($9 \cdot 10^5$ cfu/g) on the 10^{th} day of storage. Storage period (time) statistically significantly affected the growth of Enterobacteriaceae in K and OD samples at each sampling point. Statistically significantly lower count of Enterobacteriaceae in all dehydrated mushroom samples were found compared to the control sample.

The application of biopolymer coating had a statistically significant contribution to the microbiological stability of the tested mushroom samples. The lowest count of Enterobacteriaceae was recorded in OD+C and OD+Z samples, in the range from <10 to 91000 cfu/g and in the range from 93000 to 200000 cfu/g, respectively. In osmotically dehydrated mushrooms with zein coating (OD+Z) statistically significant increase in Enterobacteria count occurred after 10 days of storage. In the OD+C sample, a statistically significant decrease in the number of Enterobacteria was observed after 4 days of storage, while after 7 days the values fell below the detection limit, which revealed chitosan's significant inhibitory effects on the growth of these microorganisms. Chitosan again manifested antimicrobial activity compared to the zein-coated mushroom samples. A similar effect was achieved by Wang et al. (2021) when applying chitosan/zein films loaded with lemon essential oil on mushrooms (*A. bisporus*). There are many other studies that manifest antimicrobial properties of biopolymer films/coatings. Louis et al. (2021) achieved antimicrobial activity using alginate-based coating during the 16-day shelf-life of mushrooms (*A. bisporus*). Han et al. (2015) applied poly (lactic acid) biopolymer film for mushroom quality preservation.

Quality of burek stuffed with filling prepared from the treated mushrooms

According to the achieved antimicrobial effect, accompanied with the lowest weight loss, the osmotically dehydrated mushroom samples with chitosan coating (OD+C) were selected as an

ingredient for filling in a traditional pie-like baked product-burek.

Nutritional composition and energy value

The nutritional composition and energy value of burek prepared with fresh mushrooms (control), with osmotically dehydrated mushrooms (OD) and osmotically dehydrated mushrooms coated with chitosan (OD+C) were presented in Table 4. The obtained results indicated uniform values for fat, protein and salt content in all tested samples. The only significant difference was observed in total sugar and carbohydrate contents in the samples of burek prepared with osmotically dehydrated mushrooms (with and without chitosan coating) in relation to the control. Significantly higher amount of carbohydrates in molasses-treated mushrooms resulted in a significantly higher energy value of burek, in comparison to the control. The effect of chitosan coating on the chemical composition of the burek was not statistically significant compared to the control sample.

Sensory evaluation

Figure 2a represents the appearance of mushrooms, prepared according to the described methodology, and as such prepared to be a filling for burek, while in Figure 2b burek, as a final product, was shown. Figure 2b shows the external appearance and well as cross-sections of the prepared burek. In each figure label, 1 refers to the control sample (K), label 2 refers to (burek with) osmotically treated mushrooms (OD) and label 3 refers to (burek with) osmotically treated mushrooms with chitosan coating (OD+C). The QDA results of burek samples are depicted in Fig. 3, Panel members evaluated product appearance (shape and volume) and top surface appearance as typical, for all three sam-

Table 3.
Microbiological analysis for mushrooms (control mushroom (K), osmotically dehydrated mushroom (OD), osmotically dehydrated mushroom coated with zein (OD+Z) and osmotically dehydrated mushroom coated with chitosan (OD+C)) in different storage periods

Storage period (days)	Total counts							
	Yeasts and molds (cfu/g)				Enterobacteriaceae (cfu/g)			
	K	OD	OD+C	OD+Z	K	OD	OD+C	OD+Z
1	4000 ± 100^{g}	$500\pm100^{b,c}$	$500\pm100^{b,c}$	$<100\pm0^{a}$	$310000\pm10000^{e,f}$	190000 ± 10000^{d}	91000 ± 1000^{b}	93000 ± 1000^{b}
2	4000 ± 200^{g}	$500\pm0^{b,c}$	$300\pm100^{a,b}$	$<100\pm0^{a}$	390000 ± 20000^{g}	270000 ± 20000^{e}	91000 ± 2000^{b}	$120000\pm10000^{b,c}$
4	4000 ± 100^{g}	$900\pm100^{d,e}$	$<100\pm0^{a}$	$800\pm100^{c,d}$	570000 ± 20000^{f}	260000 ± 10000^{e}	1100 ± 100^{a}	$130000\pm10000^{b,c}$
7	4300 ± 200^{g}	$1000\pm100^{d-f}$	$<100\pm0^{a}$	$1000\pm100^{d-f}$	730000 ± 30000^{i}	$340000\pm30000^{f,g}$	$<10\pm0^{a}$	$170000\pm10000^{c,d}$
10	4800 ± 200^{h}	1300 ± 200^{f}	$<100\pm0^{a}$	$1200\pm200^{e,f}$	900000 ± 30000^{j}	$350000\pm20000^{f,g}$	$<10\pm0^{a}$	200000 ± 20000^{d}

$^{a-j}$ *Different letters in superscript for each data set regarding each microbiological response indicate statistically significant difference among means, at the level of significance of p<0.05 (based on post-hoc Tukey's HSD test)*

Table 4.
Nutritional composition and energy value of burek prepared with fresh mushrooms (control), osmotically dehydrated mushrooms (OD) and osmotically dehydrated mushrooms coated with chitosan (OD+C)

Parameter	Control	OD	OD+C
Fat content (%)	12.38 ± 0.58^{a}	11.53 ± 0.55^{a}	10.18 ± 0.45^{a}
Protein content (%)	5.99 ± 0.04^{a}	6.36 ± 0.30^{a}	6.63 ± 0.32^{a}
Total carbohydrates (%)	23.30 ± 0.30^{b}	43.20 ± 2.2^{a}	39.80 ± 1.89^{a}
Total sugar (%)	1.44 ± 0.05^{b}	5.36 ± 0.24^{a}	5.21 ± 0.22^{a}
Salt content (g/100g)	1.32 ± 0.04^{a}	1.69 ± 0.06^{a}	1.86 ± 0.06^{a}
Energy value (kcal/100g)	228.40 ± 13.42^{b}	302.01 ± 12.50^{a}	277.34 ± 13.50^{a}

$^{a-b}$*Different letters in superscript in a row indicate statistically significant difference between values, at the level of significance of p<0.05 (based on post-hoc Tukey HSD test)*

Figure 2a. The appearance of mushrooms prepared for burek filling: 1. Control sample (K); 2. Osmotically dehydrated mushrooms (OD); 3. Osmotically dehydrated mushrooms with chitosan coating (OD+C)

Figure 2b. The external appearance and cross-sections of the prepared burek 1. Control burek sample (K); 2. Burek with osmotically dehydrated mushrooms (OD); 3. Burek with osmotically dehydrated mushrooms coated with chitosan (OD+C)

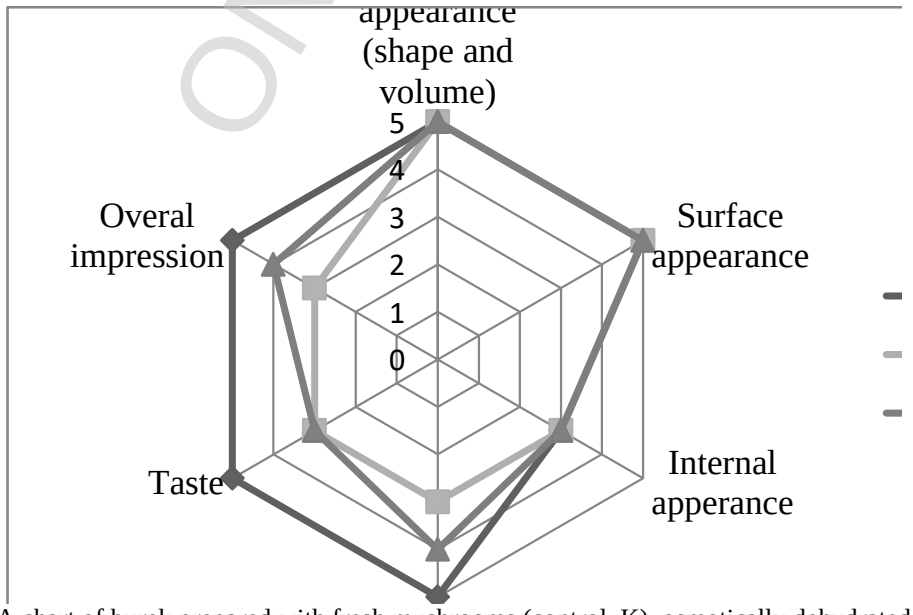

Figure 3. QDA chart of burek prepared with fresh mushrooms (control, K), osmotically dehydrated mushrooms (OD) and osmotically dehydrated mushrooms coated with chitosan (OD+C)

ples. In terms of internal appearance, panellists observed a soft, fluffy structure but with the occurrence of undesirable sticky, merged layers in all three examined samples. The obtained results indicate that the OD burek sample achieved the weakest sensory acceptance concerning control (K) and OD+C samples. Further, the control group K and OD+C were the most preferred groups based on odour and overall impression.

CONCLUSIONS

In this paper, the positive effects of osmotic dehydration and biopolymer coatings on maintainning the quality and prolonging the shelf life of mushrooms *(A. bisporus)* were confirmed. The osmotically dehydrated (OD) mushrooms in molasses, as the hypertonic solution, were minimally processed, very similar to the fresh ones, and exerted prolonged shelf life. The obtained osmotic (semi) products (OD mushrooms) had reduced moisture content, which contributed to their stability. A step further in increasing the stability of the mushroom semiproduct was the application of biopolymers (zein and chitosan).

The application of biopolymer coatings was an effective way to control the microenvironment by reducing the rate of respiratory metabolism and inhibiting microbial growth. Chitosan proved to be more efficient in preserving quality compared to zein.

The baking performance of treated mushrooms as fillings in a pie-like, stuffed, layered pastry (burek) was evaluated. The performances of untreated mushrooms (in the control burek), OD mushrooms and chitosan-coated OD mushrooms were compared. Results proved that control burek and burek samples with osmotically dehydrated mushrooms coated with chitosan were the most preferred groups based on odour and overall impression.

In conclusion, most studies on edible coatings for food packaging applications have been limited to laboratory investigations. A further step should be scaling up to commercial applications.

ACKNOWLEDGEMENTS

This paper is a result of the research within the project TR 31055 (451-03-9/2021-14/200134), supported by the Ministry of Education, Science and Technology, Republic of Serbia.

REFERENCES

Alemán, A., González, F., Arancibia, M. Y., López-Caballero, M. E., Montero, P., & Gómez-Guillén, M. C. (2016). Comparative study between film and coating packaging based on shrimp concentrate obtained from marine industrial waste for fish sausage preservation. *Food Control*, *70*, 325-332. https://doi.org/10.1016/j.foodcont.2016.06.007

Aziz, T., Hassan, M., Talukder, F. U., & Rahman, M. (2021). Effects of different concentrations of chitosan on shelf life and quality of banana fruit. *International Journal of Horticultural Science and Technology*, *8*(1), 1-12. https://10.22059/ijhst.2020.309397.387

Bico, S. L. S., Raposo, M. F. J., Morais, R. M. S. C., & Morais, A. M. M. B. (2009). Combined effects of chemical dip and / or carrageenan coating and / or controlled atmosphere on quality of fresh-cut banana. *Food Control*, *20*(5), 508-514. https://doi.org/10.1016/j.foodcont.2008.07.017

Cardwell, G., Bornman, J. F., James, A. P., & Black, L. J. (2018). A review of mushrooms as a potential source of dietary vitamin D. *Nutrients*, *10*(10), 1498. https://doi.org/10.3390/nu10101498

Castelló, M. L., Igual, M., Fito, P. J., & Chiralt, A. (2009). Influence of osmotic dehydration on texture, respiration and microbial stability of apple slices (var. Granny Smith). *Journal of Food Engineering*, *91*(1), 1-9. https://doi.org/10.1016/j.jfoodeng.2008.07.025

da Silva Júnior, A. F., da Silva, W. P., de Farias Aires, J. E., Aires, K. L. C. A. F., & de Castro, D. S. (2017). Osmotic dehydration kinetics of banana slices considering variable diffusivities and shrinkage. *International Journal of Food Properties*, *20*, 1313-1325. https://doi.org/10.1080/10942912.2016.1209215

Diamantopoulou, P., & Philippoussis, A. (2015). *Cultivated mushrooms: Preservation and processing. Handbook of vegetable preservation and processing* (pp. 495-525). Florida: CRC Press.

Divya, K., Smitha, V., & Jisha, M. S. (2018). Antifungal, antioxidant and cytotoxic activities of chitosan nanoparticles and its use as an edible coating on vegetables. *International Journal of Biological Macromolecules*, *114*, 572-577. https://doi.org/10.1016/j.ijbiomac.2018.03.130

Gholami, R., Ahmadi, E., & Farris, S. (2017). Shelf-life extension of white mushrooms *(Agaricus bisporus)* by low temperatures conditioning, modified atmosphere, and nanocomposite packaging material. *Food Packaging and Shelf Life*, *14*, 88-95. https://doi.org/10.1016/j.fpsl.2017.09.001

Han, L., Qin, Y., Liu, D., Chen, H., Li, H., & Yuan, M. (2015). Evaluation of biodegradable film packaging to improve the shelf-life of *Boletus edulis* wild edible mushrooms. *Innovative Food Science and Emerging Technologies*, *29*, 288-294. http://dx.doi.org/10.1016/j.ifset.2015.04.008

Hossain, M. S., & Iqbal. A. (2016). Effect of shrimp chitosan coating on postharvest quality of banana *(Musa sapientum* L.*)* fruits. *International Food Research Journal*, *23*(1), 277-283.

Hozová, B., Kukurová, I., Turicová, R., & Dodok, L. (2002). Sensory Quality of Stored Croissant-Type Bakery Products. *Czech Journal of Food Science*, *20*(3), 105-112. https://doi.org/10.17221/3519-CJFS

Huang, Q., Qian, X., Jiang, T., & Zheng, X. (2019). Effect of chitosan and guar gum based composite edible coating on quality of mushroom *(Lentinus edodes)* during postharvest storage. *Scientia Horticulturae*, *253*, 382-389. https://doi.org/10.1016/j.scienta.2019.04.062

Ibukunoluwa, F. O., Park, J. J., Lee, J. J., & Lee, W. Y. (2019). Combined effect of chitosan coating and modified atmosphere packaging on fresh-cut cucumber. *Food Science & Nutrition*, *7*(3), 1043-1052, https://doi.org/10.1002/fsn3.937

ISO (2007). *Sensory analysis – general guidance for the design of test rooms*, AMENDMENT 1. In: ISO 8589. Geneva, Switzerland: International Organization for Standardization.

Jiang, T. (2013). Effect of alginate coating on physico-chemical and sensory qualities of button mushrooms *(Agaricus bisporus)* under a high oxygen modified atmosphere. *Postharvest Biology and Technology*, *76*, 91-97. https://doi.org/10.1016/j.postharvbio.2012.09.005

Jiang, T., Feng, L., & Li, J. (2012). Changes in microbial and postharvest quality of shiitake mushroom (Lentinus edodes) treated with chitosan–glucose complex coating under cold storage. *Food Chemistry*, *131*(3), 780-786. https://doi.org/10.1016/j.foodchem.2011.08.087

Karimirad, R., Behnamian, M., & Dezhsetan, S. (2018). Development and characterization of nano biopolymer containing cumin oil as a new approach to enhance antioxidant properties of button mushroom. *International Journal of Biological Macromolecules*, *113*, 662-668. https://doi.org/10.1016/j.ijbiomac.2018.02.043

Kaya, M., Česonienė, L., Daubaras, R., Leskauskaitė, D., & Zabulionė, D. (2016). Chitosan coating of red kiwifruit *(Actinidia melanandra)* for extending of the shelf life. *International Journal of Biological Macromolecules*, *85*, 355-360. https://doi.org/10.1016/j.ijbiomac.2016.01.012

Khan, M. R. (2012). Osmotic dehydration technique for fruits preservation – A review. *Pakistan Journal of Food Sciences*, *22*(2), 71-85. https://doi.org/10.1007/s13197-012-0659-2

Kumar, A., Singh, M., & Singh, G. (2013). Effect of different pretreatments on the quality of mushrooms during solar drying. *Journal of Food Science & Technology*, *50*(1), 165-170. https://doi.org/10.1007/s13197-011-0320-5

Kumar, P., Sethi, S., Sharma, R. R., Srivastav, M., & Varghese, E. (2017). Effect of chitosan coating on postharvest life and quality of plum during storage at low temperature. *Scientia Horticulturae*, *226*, 104-109. https://doi.org/10.1016/j.scienta.2017.08.037

Kumarihami, H. M. P. C., Kim, J. G., Kim, Y.-H., Lee, M., Lee, Y.-S., Kwack, Y.-B., & Kim, J. (2021). Preharvest application of chitosan improves the postharvest life of 'Garmrok' Kiwifruit through the modulation of genes related to ethylene biosynthesis, CellWall Modification and Lignin Metabolism. *Foods*, *10*, 373. https://doi.org/10.3390/foods10020373

Liu, Q., Cui, X., Song, Z., Kong, W., Kang, Y., Kong, W., & Ng, T. B. (2021). Coating shiitake mushrooms *(Lentinus edodes)* with a polysaccharide from *Oudemansiella radicata* improves product quality and flavor during postharvest storage. *Food Chemistry*, *352*, 129357. https://doi.org/10.1016/j.foodchem.2021.129357

Louis, E., Villalobos-Carvajal, R., Reyes-Parra, J., Jara-Quijada, E., Ruiz, C., Andrades, P., Gacitúa, J., & Beldarraín-Iznaga, T. (2021). Preservation of mushrooms *(Agaricus bisporus)* by an alginate-based-coating containing a cinnamaldehyde essential oil nanoemulsion. *Food Packaging and Shelf Life*, *28*, 100662. https://doi.org/10.1016/j.fpsl.2021.100662

Maqbool, M., Ali, A., Alderson, P. G., Zahid, N., & Siddiqui, Y. (2011). Effect of a novel edible composite coating based on gum arabic and chitosan on biochemical and physiological responses of banana fruits during cold storage. *Journal of Agricultural and Food Chemistry*, *59*, 5474-5482. https://doi.org/10.1021/jf200623m

Mohebbi, M., Ansarifar, E., Hasanpour, N., & Amiryousefi, M. R. (2012). Suitability of aloe vera and gum tragacanth as edible coatings for extending the shelf life of button mushroom. *Food and Bioprocess Technology*, *5*(8), 3193-3202. http://dx.doi.org/10.1007/s11947-011-0709-1

Pacheco-Angulo, H., Herman-Lara, E., García-Alvarado, M. A., & Ruiz-López, I. I. (2016). Mass transfer modeling in osmotic dehydration: equilibrium characteristics and process dynamics under variable solution concentration and convective boundary. *Food and Bioproducts Processing*, *97*, 88-99. https://doi.org/10.1016/j.fbp.2015.11.002

Perdones, A., Sánchez-González, L., Chiralt, A., & Vargas, M. (2012). Effect of chitosan-lemon essential oil coatings on storage-keeping quality of strawberry. *Postharvest Biology and Technology*, *70*, 32-41. https://doi.org/10.1016/j.postharvbio.2012.04.002

Phisut, N. (2012). Factors affecting mass transfer during osmotic dehydration of fruits. *International Food Research Journal*, *19*(1), 7-18. https://doi.org/10.12691/ajfst-7-3-2

Pravilnik. (2018). Pravilnik o deklarisanju, označavanju i reklamiranju hrane. *Službeni glasnik RS, 19/2017, 16/2018, 17/2020, 118/2020, 17/2022, 23/2022 i 30/2022*.

Pravilnik. (1988). Pravilnik o metodama fizičkih i hemijskih analiza za kontrolu kvaliteta žita, mlinskih i pekarskih proizvoda, testenina i brzo smrznutih testa, *Sl. list SFRJ, 74/88; methods 2.3., 2.4., 2.6., 2.9.*

Qiu, L., Zhang, M., Tang, J., Adhikari, B. & Cao, P. (2019). Innovative technologies for producing and preserving intermediate moisture foods: A review. *Food Research International*, *116*, 90-102. https://doi:10.1016/j.foodres.2018.12.055

Rangaraj, V. M., Rambabu, K., Banat, F., & Mittal, V. (2021). Effect of date fruit waste extract as an antioxidant additive on the properties of active gelatin films. *Food Chemistry*, *355*, 129631. https://doi.org/10.1016/j.foodchem.2021.12963

Rathore, H., Prasad, S., & Sharma, S. (2017). Mushroom nutraceuticals for improved nutrition and better human health: A review. *PharmaNutrition*, *5*(2), 35-46. https://doi.org/10.1016/j.phanu.2017.02.001

Ruan, C-Q., Kang, X., & Zeng, K. (2022). Preparation of water-soluble dialdehyde cellulose enhanced chitosan coating and its application on the preservation of mandarin fruit. *International Journal of Biological Macromolecules*, *203*, 184-194.

https://doi.org/10.1016/j.ijbiomac.2022.01.010

Samsudin, N. I. P., & Abdullah, N. (2019). Edible mushrooms from Malaysia; a literature review on their nutritional and medicinal properties. *International Food Research Journal*, *26*(1), 11-31. http://psasir.upm.edu.my/id/eprint/67288/1/%282%29.pdf

SRP ISO 21527-2. (2011). Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and molds — Part 2: Colony count technique in products with water activity less than or equal to 0,95.

SRPS EN ISO 11290-2. (2017). Microbiology of the food chain – Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2: Enumeration method.

SRPS EN ISO 21528-2. (2017). Microbiology of the food chain – Horizontal method for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count technique.

SRPS EN ISO 6579-1. (2017). Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp.

SRPS ISO 16649-2. (2008). Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli – Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.

SRPS ISO 5492. (2000). Sensory analysis – Vocabulary.

SRPS ISO 937. (1992). Meat and meat products – Determination of nitrogen content.

Sucharitha, K. V., Beulah, A. M., & Ravikiran, K. (2018). Effect of chitosan coating on storage stability of tomatoes (Lycopersicon esculentum Mill). *International Food Research Journal*, *25*(1), 93-99.

Šarić, Lj., Filipčev, B., Šimurina, O., Plavšić, D., Šarić, B., Lazarević, J., & Milovanović, I. (2016). Sugar beet molasses: Properies and application in osmotic dehydration of fruits and vegetables. *Food and Feed Research*, *43*(2), 135-144. https://doi.org/10.5937/FFR1602135Š

Šuput D., Filipović V., Lončar B., Nićetin M., Knežević V., Lazarević J., & Plavšić D. (2020). Modeling of mushrooms *(Agaricus bisporus)* osmotic dehydration process in sugar beet molasses. *Food and Feed Research*, *47*(2), 175-187. https://doi.org/10.5937/ffr47-28436

Šuput, D., Lazić, V., Popović, S., & Hromiš, N. (2015). Edible films and coatings– sources, properties and application. *Food and Feed Research*, *42*(1), 11-22. https://doi.org/10.5937/FFR1501011S

Vieira, J. M., Flores-López, M. L., de Rodríguez, D. J., Sousa, M. C., Vicente, A. A. & Martins, J. T. (2016). Effect of chitosan-Aloe vera coating on postharvest quality of blueberry *(Vaccinium corymbosum)* fruit. *Postharvest Biology and Technology*, *116*, 88-97. https://doi.org/10.1016/j.postharvbio.2016.01.011

Wang, X., Sun, Y., Liu, Z., Huang, X., Yi, F., Hou, F., & Zhang, F. (2021). Preparation and characterization of chitosan/zein film loaded with lemon essential oil: Effects on postharvest quality of mushroom *(Agaricus bisporus)*. *International Journal of Biological Macromolecules*, *192*, 635-643. https://doi.org/10.1016/j.ijbiomac.2021.10.068

Wrona, M., Bentayeb, K., & Nerín, C. (2015). A novel active packaging for extending the shelf-life of fresh mushrooms *(Agaricus bisporus)*. *Food Control*, *54*, 200-207. https://doi.org/10.1016/j.foodcont.2015.02.008

Xin, Y., Chen, F., Lai, S., & Yang, H. (2017). Influence of chitosan-based coatings on the physicochemical properties and pectin nanostructure of Chinese cherry. *Postharvest Biology and Technology*, *133*, 64-71. https://doi.org/10.1016/j.postharvbio.2017.06.010

Xu, Y., Tian, Y., Ma, R., Liu, Q., & Zhang, J. (2016). Effect of plasma activated water on the postharvest quality of button mushrooms, Agaricus bisporus. *Food Chemistry*, *197*, 436-444. https://doi.org/10.1016/j.foodchem.2015.10.144

Zhang, K., Pu, Y-Y., & Sun, D-W. (2018). Recent advances in quality preservation of postharvest mushrooms *(Agaricus bisporus)*: A review. *Trends in Food Science & Technology*, *78*, 72-82. https://doi.org/10.1016/j.tifs.2018.05.012

Zhang, Y., Che, X., Zhang, H., Shi, N., Li, C., Chen, Y., & Kong, W. (2015). Zein-based films and their usage for controlled delivery: Origin, classes and current landscape. *Journal of Controlled Release*, *206*, 206-219. https://doi.org/10.1016/j.jconrel.2015.03.030

UTICAJ BIOPOLIMERNIH PREMAZA NA STABILNOST SKLADIŠTENJA OSMOTSKI DEHIDRIRANIH PEČURAKA

Danijela Z. Šuput[*1], Vladimir S. Filipović[1], Biljana Lj. Lončar[1], Milica R. Nićetin[1], Violeta M. Knežević[1], Jasmina M. Lazarević[2], Dragana V. Plavšić[2], Senka Z. Popović[1], Nevena M. Hromiš[1]

[1]Univerzitet u Novom Sadu, Tehnološki fakultet Novi Sad, 21000 Novi Sad, Bulevar cara Lazara 1, Srbija
[2]Univerzitet u Novom Sadu, Naučni institut za prehrambene tehnologije u Novom Sadu, 21000 Novi Sad, Bulevar cara Lazara 1, Srbija

Sažetak: Glavni cilj ovog istraživanja je primena biopolimernih premaza na osmotski dehidrirane pečurke i praćenje njihovog kvaliteta tokom skladištenja. Pečurke su osmotski dehidrirane u melasi šećerne repe (80% suve materije) pod optimizovanim uslovima koji su prethodno objavljeni drugde (45 °C tokom 5 sati). Izabrana su dva različita biopolimera: hitozan, koji je polisaharidni biopolimer, i zein, proteinski biopolimer. Kao kontrolni uzorak odabran je netretiran uzorak pečuraka. U uzorcima pečuraka je određen sadržaj šećera i proteina, gubitak vode i mikrobiološki profil. Prinos šećera je bio najuočljiviji u osmotski dehidriranim uzorcima pečuraka u poređenju sa kontrolnim uzorkom, zbog upotrebe melase kao hipertoničnog rastvora. Doprinos primene biopolimernih premaza sadržaju šećera i proteina bio je zanemarljiv. Najveći doprinos na smanjenje gubitka vlage i mikrobiološkoj stabilnosti tj. bolju skladišnu stabilnost pečuraka imao je biopolimerni premaz od hitozana. Zbog toga je uzorak pečurki sa hitozanskim omotačem izabran za dalje ispitivanje njegove primenljivosti kao punjenja u u tradicionalnom pekarskom proizvodu bureku. Burek je pripremljen od svežih pečuraka, osmotski tretiranih pečuraka i osmotski tretiranih pečuraka premazanih hitozanom. Senzorna ocena pokazala je da su kontrolni uzorak bureka i uzorak bureka sa osmotski dehidriranim pečurkama premazanim hitozanom bili najprihvatljiviji uzorci na osnovu mirisa i ukupnog utiska.

Ključne reči: *melasa, pečurke, zein, hitozan, stabilnost skladištenja, burek, senzorska svojstva*

Received: *10 January 2022/* ***Received in revised form:*** *12 May 2022/* ***Accepted:*** *8 June 2022*

Available online: *June 2022*