TWO-LAYER COATING BASED ON CHITOSAN FOR DRY FERMENTED SAUSAGE PRESERVATION

DVOSLOJNI OMOTAČ NA BAZI HITOZANA ZA ZAŠTITU FERMENTISANE SUVE KOBASICE

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ABSTRACT

In this paper, the application of a biopolymer coating based on chitosan was tested. The optimized coating was formed as a two-layer coating, wherein the first layer (which is in contact with the product) was formed of chitosan with emulsified caraway essential oil as an active component, whereas the second layer was formed of chitosan with the addition of beeswax to optimize the moisture barrier of the coating. As a substrate, the traditional dry fermented sausage Petrovská klobása was selected. The coating was applied to the sausage after drying. During two months of storage under controlled conditions, the influence of the coating on the loss of moisture, the oxidative stability and the sensory characteristic of aroma of the control sausage (without the coating) and sausage with the coating was monitored. The results obtained indicate a significant contribution of the applied dual layer chitosan-based coating to the preservation of the sausage quality parameters analyzed.

Key words: biopolymer coating, chitosan, caraway essential oil, beeswax, fermented dry sausage, quality parameters

REZIME

U ovom radu je ispitana mogućnost primene biopolimernog omotača na bazi hitozana. Optimizovani omotač je formiran kao dvoslojni, gde je prvi sloj koji je u kontaktu sa proizvodom formiran od hitozana sa emulgovanim etarskim uljem kima, dok je drugi sloj formiran od hitozana uz dodatak pčelinjeg voska. Kao supstrat je odabrana tradicionalna suva fermentisana kobasica Petrovská klobása. Omotač je nanet na kobasicu nakon završetka sušenja i tokom dva meseca skladištenja kobasice u kontrolisanim uslovima, praćen je uticaj navedenog omotača na gubitak vlage kobasice, oksidativnu stabilnost i senzorsko svojstvo arome kontrolne kobasice (bez omotača) i kobasice sa omotačem.

Sadržaj vlage u kobasici tokom skladištenja je opadao sa početnih 34,44% na kraju postupka sušenja na 22,59%, nakon mesec dana i 19,36% nakon dva meseca skladištenja. U kobasici sa omotačem, ova vrednost je iznosila 25,09% nakon mesec dana i 22,57% nakon dva meseca skladištenja. Vrednost TBARS u kobasici na kraju sušenja je iznosila 0,18 mg/kg i tokom skladištenja je rasla, što je verovatno posledica oksidacije lipida. Nakon mesec dana skladištenja, TBARS vrednost u kontrolnoj kobasici je iznosio 0,87 mg/kg, dok je u kobasici sa omotačem ova vrednost iznosila 0,23 mg/kg. Do kraja drugog meseca skladištenja vrednost TBARS ostao je niži u kobasici sa omotačem. Takođe, senzorske ocene za aromu kobasice bile su značajno više za kobasicu sa omotačem u odnosu na kontrolnu kobasicu, tokom ukupnog perioda skladištenja.

Dobijeni rezultati ukazuju na značajan doprinos primenjenog dvoslojnog omotača na bazi hitozana na očuvanje ispitivanih parametara kvaliteta suve fermentisane kobasice.

Ključne reči: biopolimerni omotač, hitozan, etarsko ulje kima, pčelinji vosak, fermentisana suva kobasica, parametri kvaliteta

INTRODUCTION

Reducing the amount of marketed packaging waste has become an imperative stipulated in the European Directive (EU) 2015/720 and the concomitant legal implementation frameworks in different EU countries. This issue can be addressed in various manners such as the recycling, reuse and use of biodegradable and biopolymer materials. The possibility of using biopolymer-based materials in the protection of nutritive, sensory, technological and other food properties is intensively studied in order to expand the scope of their application (Siracusa *et al.*, 2008; Lazić and Popović, 2015).

The use of edible films in the food industry is currently limited. Most hydrocolloid films cannot be used for food products with high surface water activity (i.e. $a_w > 0.94$) because they are subjected to degradation, melting or swelling in contact with moisture, which results in a loss of barrier properties. The use of edible films is mainly limited to foods with low or medium moisture contents, as well as frozen products. Generally, edible films are more sensitive to the touch

of wet hands as well as changes in ambient conditions (in particular, changes in the relative humidity). The use of edible coatings should be accompanied by the use of additional packaging materials, which would preserve the edible protective film as well as the integrity and safety of such a package (Galić, 2009; Falguera *et al.*, 2011).

Chitosan, as a semi-natural biopolymer, has been extensively studied for edible film application. Relative to the use of chitosan in the production of edible films in the food industry, this biopolymer possesses the following favourable properties: it is approved as an additive in the food industry in many countries, has the ability to form films with good gas barrier properties, demonstrates antimicrobial and antioxidant properties, three functional groups enable different modifications of the molecules in order to improve the properties, the source is a renewable raw material, which is also a waste material from the food industry, the resulting edible films can be consumed together with the product, or are rejected as biodegradable packaging (Elsabee and Abdou, 2013; Chhabra, 2004; Hammond, 2004).

This paper will contribute to expanding the application scope of biopolymer coatings based on chitosan with the addition of active and functional components. The optimized coating was formed as a two-layer coating, wherein the first layer (which is in contact with the product) was formed of chitosan with emulsified caraway essential oil as an active component, whereas the second layer was formed of chitosan with the addition of beeswax to optimize the moisture barrier of the coating. As a substrate, the traditional dry fermented sausage Petrovská klobása (Petrovac sausage) was selected. The coating was applied to the sausage after drying. During two months of storage under controlled conditions, the influence of the coating on the loss of moisture, the oxidative stability and the sensory characteristic of aroma of the control sausage (without the coating) and sausage with the coating was monitored. The results obtained indicate a significant contribution of the applied dual layer chitosan-based coating to the preservation of the sausage quality parameters analyzed.

MATERIAL AND METHOD

The sample sausages were prepared from lean pork meat and fat in a ratio of 80:20, also containing additional spices. The preparation process was described in details in Krkić et al. (2012), whereas the only modification to the process described was the inoculation of sausages with *Staphylococcus xylosum*.

Chitosan film forming emulsions were prepared as described in Hromiš et al. (2016). The coating applied was designed in two layers for different uses. The chitosan powder (Sigma-Aldrich Chemical Co., St. Louis, Missouri, USA) was dissolved in acetic acid (1% vol.; Proanalitica, Belgrade, Serbia) to reach a chitosan mass per volume ratio of 10 kg/m³. For the synthesis of the first coating layer, caraway essential oil (Herba D.O.O., Belgrade, Serbia) was added (in a volume concentration of 1 %), in addition to Tween 20 (0.5 % vol, Superlab, Belgrade, Serbia), to the chitosan solution. For the synthesis of the second coating layer, beeswax (from a local beekeeper) was added to the chitosan solution to obtain the film-forming emulsion with a beeswax mass per volume ratio of 144 kg/m³.

At the end of drying process, the produced sausages were of the following composition: 34.44±0.06 % moisture content, 27.94±0.34 % protein content, 27.81 % fat content, 2.44±0.02 % connective tissue content, 2.25±0.02 % NaCl content and 4.07±0.20 % ash content (the chemical composition of sausages was determined according to the appropriate ISO standards). After drying, the sausages were divided into two groups: control (without coating) and coated (with two-layer chitosan coating) group.

The sausages were coated with film-forming emulsions using a sponge brush. Both layers were applied in three repeated brushing/drying (at least for 1h) steps. After coating, the sausages were stored for two months at 15 °C and 75 % RH.

TBARS determination

The TBARS test was performed using the method of Bostoglou et al. (1994), with modifications according to Mandić (2007). The test procedure was described in Krkić et al. (2013) and it was performed using a spectrophotometer JENWAY 6300 (Jenway, Felsted, United Kingdom).

Sensory evaluation of aroma

Sensory evaluation was performed using a quantitative descriptive analysis (ISO 8586-1, 1993; ISO 4121, 2003; ISO 8586-2, 2008) according to the method previously described in Šojić et al. (2015), using a scale from 0 to 5, with a sensitivity threshold of 0.25 points. Each mark denotes a distinctive quality level described as follows: 5 – extraordinary, typical, optimal quality; 4 – observable deviations or insignificant quality

defects; 3 – drawbacks and defects in quality; 2 – distinct to very distinct drawbacks and defects in quality; 1 – fully changed, atypical properties, product unacceptable; 0 – visible mechanical or microbiological contamination atypical of the product.

Statistical analysis

All the determinations were carried out on three samples (three different sausages) from each group (coated and control) in duplicate (two repetitions were performed on each sausage).

A statistical analysis was carried out using OriginPro 8. All the data were presented as mean values with the standard deviation indicated (mean ± SD). The variance analysis (ANOVA) was performed, with a confidence interval of 95 % (p < 0.05), and the means obtained were compared by the Tukey test.

RESULTS AND DISCUSSION

At the end of drying process, the moisture content of dry fermented sausages should be less than 35 % (Sl. Glasnik RS, 94/2015, 2015). The moisture content of the control sausage in this experiment (Fig. 1) was 34.44 % after drying. During storage, the moisture content of the control sausages decreased from the initial value (recorded at the end of drying) to 22.59 % after a month of storage, and to 19.36 % after two months of storage. The moisture content of the coated sausage was 25.09 % after a month of storage and 22.57 % after two months of storage. During the storage period, the moisture content was higher in the coated sausages (P < 0.05), probably due to the moisture barrier properties of the coating (Hromiš et al., 2016). Decreasing the moisture loss during storage is an important contribution to the extension of sausage shelf life, followed by economic benefits. Compared to different monolayer chitosan coatings for dry fermented sausage application, the two-layer coating applied in this research produced better results than a chitosan-caraway essential oil coating (Hromiš et al., 2013), and similar results to a chitosan-caraway essential oil-beeswax coating (Hromiš et al., 2017).

Fig. 1. Moisture content (%) of the control and coated sausages during two months of storage

The TBARS test is one of the commonly used methods for the evaluation of oxidative stability of meat and meat products (Šojić et al., 2015). The TBARS values in the control and coated sausages during storage are presented in fig. 2. The TBARS value of the sausage at the end of drying was 0.18 mg/kg. The TBARS result obtained is consistent with the results reported on Petrovac sausage, as well as other sausages of this type (Šojić et

al., 2015a; Šojić et al., 2016; Ruiz-Capillas et al., 2012; Corral et al., 2013). During storage, the TBARS value increased in both the control and coated sausages, which is probably due to lipid oxidation. After a month of storage, the TBARS value in the control sausage was 0.87 mg/kg, whereas in the coated sausage this value was 0.23 mg/kg. By the end of the second month of storage, the value of TBARS remained lower in the coated sausage (1.39 mg/kg) compared to the control sausage (1.49 mg/kg) ($P < 0.05$). The results obtained are in accordance with our previous results on chitosan based coatings for dry fermented sausage application (Hromiš et al., 2017; Hromiš et al., 2013). Biopolymer films based on chitosan have already been found to slow lipid oxidation in fish and meat products, and authors attribute this effect to a combination of multiple factors. High barrier properties of chitosan films for oxygen are the most important factor. However, authors also underscore the overriding importance of the formation of stable fluorosphere of primary amino groups from chitosan and volatile aldehydes as malondialdehyde (Weist and Karel, 1992; Mohan et al., 2012), the ability of chitosan of high degree of deacetylation to scavenge free radicals (Park et al., 2004), as well as the chitosan ability to chelate metal ions (Mohan et al., 2012).

Fig. 2. TBARS value (mg/kg) of the control and coated sausages during two months of storage

Fig. 3. Sensory evaluation of the aroma of control and coated sausages during two months of storage

The sensory evaluation of the aroma of control and coated sausages after drying and during storage is shown in Fig 3. After a month of storage, the sensory marks for aroma were 2.79 for the control and 4.04 for the coated sausage, whereas, after two months of storage, the marks were 2.75 for the control and 3.64 for the coated sausage. The aroma of the coated sausage was evaluated with higher marks throughout the storage period ($P < 0.05$). The coating applied showed the significant potential for preserving sausage aroma during storage. These results are in agreement with the TBARS results, which showed a higher oxidative stability of the coated sausages.

CONCLUSION

The applied two-layer chitosan coating contributed significantly to all three tested quality parameters of the traditional dry fermented sausage *Petrovská klobása* during two months of storage under controlled conditions. The coating impeded the moisture loss from the sausages, reduced lipid oxidation and preserved the sausage aroma to a significant extent during storage. The results obtained indicate the significant potential of the tested coating for expanding the shelf life of dry fermented sausages.

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