EFFECT OF CHITOSAN-CARAWAY COATING ON COLOR STABILITY AND LIPID OXIDATION OF TRADITIONAL DRY FERMENTED SAUSAGE

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Chitosan, the second most abundant polysaccharide in nature, after cellulose, has been tested for numerous applications, among which for edible film and coating. Chitosan-based coating showed positive results for shelf life prolongation of meet products. In this paper, dry fermented sausage (Petrovská klobása) was coated with chitosan-caraway film. The effect of coating on the moisture content, color and lipid oxidation was investigated during a five-month period of storage.

The moisture content decreased rapidly during the storage and the coating did not slow down the loss of moisture. The Lightness (L*) of the sausage surface increased by the coating application, while the redness (a*) and yellowness (b*) did not change. The coated sausages showed a better color stability of the sausage core through the storage time. Also, coated sausage showed a better oxidative stability till the 60th day of storage, while this difference was not detected at the end of the storage period. Apart from slowing down sausage drying during the storage, chitosan-caraway coating was effective in preserving the sausage quality.

KEY WORDS: chitosan coating, dry fermented sausage, color stability, lipid oxidation

INTRODUCTION

Chitosan-based coating shows positive effects on meat products shelf life prolongation. The research on the storage stability of pork dipped in chitosan solution indicated that dipping method was effective in extending shelf life and preventing lipid oxidation of pork. The external redness of pork treated with chitosan remained unchanged during the storage (1). Combined chitosan-spice extracts coating showed a synergistic effect in the antimicrobial and antioxidative activity and led to a decreased moisture loss and better color in chilled meat (2). Cooked pork sausage wrapped with chitosan film or with chitosan - green tea film showed lower TBA values during twenty days of refrigerated storage (3).

The chitosan macromolecular network can serve as a carrier for essential oil. It may slow down the losses of volatile compounds of the oil and help controlled release of active compounds during an extended time (4). Caraway essential oil could serve as a safe antioxidant and antiseptic supplement in preventing deterioration of foods (5).
Petrovská klobása is a traditional dry fermented sausage produced in the area around the town of Bački Petrovac in Northern Serbia. This sausage has been produced for over 250 years, according to the original recipe, with the addition of only red-hot paprika powder, salt, crushed garlic, caraway and sugar, without any additives and preservatives (6). Petrovská klobása has a Protected Designation of Origin (PDO) according to the Serbian law (7). Considering that Petrovská klobása is produced only during winter months, in the restricted area of Bački Petrovac, the market supply has been a problem. To provide market supply over the year, shelf-life prolongation has to be considered. One of the possibilities for shelf-life prolongation is the application of chitosan based coating with the addition of an active component (7).

In this work, Petrovská klobása was produced following the original recipe, modified for the addition of starter culture (8) and after production, chitosan-caraway based coating was used in order to extend the shelf-life. The quality parameters: moisture content, pH, color changes and lipid oxidation were monitored during storage and compared between coated and uncoated sausages in order to determine the preservative effect of the coating.

EXPERIMENTAL

Material

Coating preparation. Commercial, highly viscous, chitosan from crab shells was purchased from Sigma-Aldrich Chemical (St. Louis, Missouri, USA). Caraway essential oil was purchased from the manufacturer Herba doo (Belgrade, Serbia), glacial acetic acid and Tween 20 were obtained from Superlab (Belgrade, Serbia).

Chitosan coating was prepared by dissolving chitosan powder in acetic acid (1% volume concentration). Chitosan powder was added to reach the mass per volume ratio of 4 kg·m⁻³. The solution was stirred overnight on a magnetic stirrer to dissolve chitosan. After that, caraway essential oil and wetting agent Tween 20 were added to the solution in 0.8 % and 0.4 % volume concentrations, respectively.

Sausage preparation. According to the procedure for sausage manufacturing described in PDO (Protected Designation of Origin) under Serbian law, the sausages were made from Landrace pigs, using meat from main parts of the carcasses: shoulder, loin, ham, belly, tenderloin and back fat (9). All pigs were farmed in a standard pigs production system (10).

The sausages were prepared from lean pork meat and fat in a ratio 80:20. The 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. The sausages were smoked and then dried using the previously described procedure (8, 11), with one modification being the starter culture addition. The added starter culture was commercial starter culture (Quick Starter, Lay Gewirce OHG, Germany), whose composition (Staphylococcus carnosus 25%, Staphylococcus xylosus 25%, Lactobacillus sakei 25%, Pediococcus pentosaceus 25%) is most similar to the identified profile of indigenous microflora in traditional production (9).

Experimental design. After drying, one-half of the manufactured sausages were coated with three layers of coating solution using a sponge brush (assigned as coated sausages or C sausages). Every layer was left to dry overnight before the next layer was applied. The rest of
sausages were left uncoated (assigned as uncoated sausages or U sausages). After coating, all sausages were stored in a chamber with controlled temperature and relative humidity: 15°C and 75% for five months. All properties were determined before coating, after two and five months of storage. All determinations were made on three samples from each group (coated and uncoated) in duplicate.

**Methods**

**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 the initial weight lost during drying and reported on a wet basis (Eq. 1) (12).

\[ MC = \frac{100 \times (m_1 - m_2)}{m_1} \]  

\[ [1] \]

**pH.** The pH was measured using the portable pH meter (Consort T651, Turnhout, Belgium) equipped with an insertion glass combination electrode (Mettler Toledo Greifensee, Switzerland).

**Color.** Color measurements were taken on the surface and on the core of the samples. For the core color determination, measurement was done immediately after cutting the samples, to prevent color degradation as a result of light and oxygen, in accordance with the recommendations on color determination of the American Meat Science Association (13). Sample thickness was 3 cm.

Color was studied in the CIE \(L^*a^*b^*\) color space and described by the 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 and 2° standard observer angle were used and an 8 mm in the aperture of the instrument for measurement. Before each set of measurements, the instrument was calibrated using a white ceramic tile (CR-A43).

**Determination of fatty acids ratio.** The method of Folch, Lees, & Stanley (14) was used for the extraction of lipids from sausages. After extraction, the extract was put into a test tube and dissolved in 2.4 cm³ of \(n\)-hexane. An aliquot (0.6 cm³) of 2 mol/dm³ methanolic KOH solution was added. The tube was capped and vigorously shaken for 20 s and allowed to boil for one minute in water bath at 70°C. After that, 1.2 cm³ of 1 mol/dm³ HCl was added and gently stirred. After the phase separation, 3 cm³ of \(n\)-hexane were added and the upper phase containing the fatty acid methyl esters was decanted and dissolved in \(n\)-hexane to 5 cm³. Finally, 1 µL of the a Perkin–Elmer Varian, series 1400 gas chromatograph fitted with a packed column (3 m x 3.0 mm, stationary phase GP 10% SPTM-2330 on inert carrier 100/120 Chromosorb WAW) and flame ionization detection was used (Perkin-Elmer, Waltham, Massachusetts, USA). The temperature of both the injection port and the detector was 250°C. The carrier gas was \(N_2\), with flow rate of 20 mL/min. The sample volume was 2.0 µL. The identification of the fatty acid methyl esters was done by comparison of the retention times of peaks in the sample with those of standard pure compounds (Sigma-Aldrich Chemical, St. Louis, Missouri, USA). Fatty acids methyl esters were quantified as percentage of total methyl esters. The ratio of unsaturated to saturated fatty acids (UFA/SFA) was determined.
Determination of TBARS. The TBARS (2-thiobarbituric acid reactive substances) test was performed using the method of Botsoglou, Fletouris, Papageorgiou, Vassilopoulos, Mantis & Trakatellis (15), with modifications. The TCA solution (10 %) was added to the sample and extraction was performed in ultrasonic bath XUB 12 (Grant Instruments, Cambridge, UK) (16) and the determination was carried out on a Jenway 6300 spectrophotometer (Jenway, Felsted, United Kingdom). The TBARS values were expressed as milligrams of malondialdehyde per kilogram of sample.

Statistical analysis

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

RESULTS AND DISCUSSION

The moisture contents, as one of the quality parameters of the fermented dry sausages, according to the Serbian Regulation of quality and other requirements for meat products (17), are presented in Fig. 1. This type of sausage must meet the requirement that the maximum moisture content does not exceed 35%. After reaching 35%, further moisture loss is undesirable because it affects sensory quality and sausage appears over dry, tough and wrinkled. During storage, the moisture content decreased intensively and to the 60th day of storage it reached 19.80% in U and 20.71% in C (Fig. 1). To the end of the storage period of 150 days, the moisture content reached 16.13% in U and 16.66 % in C. Even though there are some differences between the compared sausages in moisture content during the storage, the coating with chitosan-caraway layer could not be considered as a protection from drying. The changes in the pH values were identical in both U and C sausages, increasing from 4.96 in the end of production to 5.29 in the end of storage period (Fig. 1).

The change in the color parameters, as shown in Table 1, were observed for the parameters $a^*$ and $b^*$, determined in the sausage core and for the $L^*$ values determined in the surface. In the U sausages, redness decreased significantly through the storage period. Redness ($a^*$) is often used as an indicator of meat and meat products color stability, and it is an important indicator of color changes the during storage (18). Papadima and Bloukas (19) explained that in traditional Greek sausages the decrease of $a^*$ value was caused by oxidation of nitrosyl myoglobin. This process was accelerated by increased salt content of the product because the salt acted as pro-oxidant (20). The decrease of the red color ($a^*$) of cut surface sausages could also be caused by the oxidation of components of red spice paprika. This decrease was not determined in the C sausages. The C sausages preserved redness through the all 150 days of storage. It was found that the increase in the content of NaCl (that follows the decrease in the moisture content) reduces the amount of yellow color, which is in agreement with the results obtained in this study, although this decrease was less pronounced in the C sausages (21). The coating lightness was altered by the chitosan-caraway layer. After 60 days of storage the C sausages showed a higher lightness value compared to the U sausages, but this difference was not significant at the end of storage.
Figure 1. Moisture content, MC (%) and pH during storage period of 150 days. Umc and Cmc are moisture contents of U and C sausages and UpH and CpH are pH values for U and C sausages. Vertical bars represent standard deviations.

Table 1. Color parameters: lightness L*, redness a* and yellowness b* for U and C sausages during the storage period of 150 days

<table>
<thead>
<tr>
<th>Day of storage</th>
<th>0</th>
<th>60</th>
<th>150</th>
<th>60</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td>L*</td>
<td>32,87±3,26a</td>
<td>26,72±1,65bc</td>
<td>29,89±4,50ab</td>
<td>24,98±4,29c</td>
</tr>
<tr>
<td></td>
<td>a*</td>
<td>20,53±1,18a</td>
<td>14,83±2,31b</td>
<td>7,04±1,34c</td>
<td>19,16±5,08a</td>
</tr>
<tr>
<td></td>
<td>b*</td>
<td>21,22±2,54a</td>
<td>13,69±2,93b</td>
<td>13,35±2,97b</td>
<td>16,48±4,83ab</td>
</tr>
<tr>
<td>Surface</td>
<td>L*</td>
<td>21,86±1,33c</td>
<td>23,03±1,39bc</td>
<td>23,19±1,66bc</td>
<td>28,77±2,04ab</td>
</tr>
<tr>
<td></td>
<td>a*</td>
<td>7,12±1,93</td>
<td>5,64±1,22</td>
<td>5,30±1,50</td>
<td>4,19±0,56</td>
</tr>
<tr>
<td></td>
<td>b*</td>
<td>6,05±1,47</td>
<td>6,55±0,64</td>
<td>6,64±1,22</td>
<td>4,77±0,16</td>
</tr>
</tbody>
</table>

Different letters abc within the same row mark significantly different means with 95% probability (p < 0.05).

Fig. 2 shows TBARS and the ratio UFA/SFA. The obtained values for malondialdehyde in Petrovská klobása were similar to some reports (11, 18, 22), but still higher than the content in some similar products (23, 24).
Figure 2. MDA content (mg/kg) and UFA/SFA ratio for U and C sausages during storage period of 150 days. Umda and Cmda are MDA contents of U and C sausages and Uufa/sfa and Cufa/sfa are UFA/SFA ratios for U and C sausages. Vertical bars represent standard deviations.

After 60 days of storage, the C sausage showed lower level of lipid oxidation than the U sausages, as is evident from the values of these two parameters. In the U sausages, the MDA (mg/kg) value increased significantly (p<0.05), from 0.34 mg/kg in the beginning of storage to 4.31 mg/kg after 60 days. This increase was less pronounced (p<0.05) in the C sausages, in which the MDA value after 60 days of storage was 3.31 mg/kg. Following the MDA values, the UFA/SFA ratio was higher in C sausages, showing that lipid oxidation was slower at this point of storage. To the end of storage period, the MDA values were 4.83 and 5.54 mg/kg in the U and C sausages, respectively, and the UFA/SFA ratio was similar in both U and C sausages. The values for the UFA/SFA ratio were similar to the results of Krkić et al. (11) and Valencia et al. (23). The Chitosan-caraway coating could not slow down lipid oxidation processes to the end of the storage period. Its contribution was visible only to the 60th day.

CONCLUSION

The color of sausage core was preserved by coating with chitosan-caraway coating during the storage of five months. At the same time, coating affected higher lightness of sausage surface, leaving the share of red and yellow color unchanged. The results suggest that the application of chitosan-caraway coating can slow down lipid oxidative changes in dry fermented sausages during the storage. Both indicators of oxidative changes, MDA and UFA/SFA ratio, showed a better oxidative stability of coated sausage to the 60th day of storage. After 60 days, to the end of storage, this effect was not visible. Among the above mentioned qualities that were preserved by the coating of the sausage, the coating was not successful in slowing down the sausage drying process during the storage.
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REFERENCES


УТИЦАЈ ОМОТАЧА НА БАЗИ ХИТОЗАНА И КИМА НА СТАБИЛНОСТ
БОЈЕ И ОКСИДАЦИЈУ ЛИПИДА ТРАДИЦИОНАЛНЕ ФЕРМЕНТИСАНЕ
КОБАСИЦЕ

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Амбалажа је пратилац прехрамбеног производа који доприноси дужем очувању производа. Тренутно у свету су најзаступљенији синтетички полимерни амбалажни материјали, произведени из необновљивих сировина и практично неразградиви (потребан јако дуг временски период) у спољашњој средини. Као альтернатива овим материјалима
развија се све већи број биополимера, који се најчешће произведе од отпада из прехрмбене индустрије, а аплицирају се у виду филмова или омотача. Један од познатијих филмогених биополимера је и хитозан, на којем се врше интензивна истраживања, а један од смерова истраживања иде у правцу примене хитозанског биофилма за паковање прехрмбених производа.

У овом раду је традиционална ферментисана кобасица (Petrovská klobása) обложена слојем биофилма на бази хитозана и кима. Утицај овог омотача на промену садржаја влаге, боје и оксидацију липида је праћен током пет месеци складиштења.

Садржај влаге је брзо опадао током складиштења и испитивани омотач није успорио губитак влаге. Светлоћа (L*) површине кобасице се повећала услед присуства омотача, док је уdeo црвене (a*) и жуте боје (b*) остао непромењен. Кобасица заштићена хитозанским омотачем показала је већу стабилност боје пресека кобасице током складиштења. Такође, кобасица са омотачем је показала бољу оксидативну стабилност до шездесетог дана складиштења, али ова разлика није била уочљива на крају периода складиштења. Изузимајући успоравање сушења кобасице током складиштења, омотач на бази хитозана и кима се показао ефикасним у заштити квалитета кобасице током складиштења и продужењу њене одрживости.

Кључне речи: хитозански омотач, сува ферментисана кобасица, стабилност боје, оксидација липида

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