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Lipid oxidative changes in chitosan-oregano coated traditional dry fermented sausage Petrovská klobása

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ABSTRACT

The effect of a chitosan coating with added essential oil of oregano (*Origanum vulgare*) on lipid oxidation of dry fermented sausage (Petrovská klobása) was investigated. Fatty acid profile, aldehyde contents and sensory analysis of odor and flavor were determined after drying and during seven months of storage.
Between coated and control sausage, a difference was observed after two months storage in fatty acid profiles (myristic, oleic and linoleic acids), but after seven months storage there was no difference. Decrease in polyunsaturated acid content was observed (from 17.25% to 15.70%), as well as an increase in total aldehydes (from 4.54 μg/g to 31.80 μg/g), due to lipid oxidation during storage. After seven months storage, the content of most aldehydes was significantly lower in coated sausage than in the control. Sensory characteristics of odor and flavor were better for coated sausage, after seven months of storage. Results suggest that chitosan–oregano coating can be successfully applied to protect dry fermented sausages from lipid oxidation.

1. Introduction

Petrovská klobása is a traditional dry fermented sausage that has been produced for over 250 years in the area of Bački Petrovac, Republic of Serbia. It is produced exclusively from pork meat and fat and spices: red hot paprika powder, salt, garlic, caraway and sugar (Tasić et al., 2012). Dry fermented sausages are susceptible to lipid oxidation that can deteriorate their sensorial properties, by generation of compounds such as n-alkenals, dienals and aldehydes, which are associated with a rancid taste and odor. Oxidation can also affect the nutritional value of food by decomposition of vitamins and unsaturated essential fatty acids (Ansorena & Astiasarán, 2004). In the food industry, different substances are used as antioxidants. Antioxidant can be defined as substance that, when present at low concentration compared to substrate, significantly delays or inhibits oxidation of that substrate (Park, Je, & Kim, 2004).

One natural antioxidant that has been tested for use in food preservation is chitosan, a polysaccharide derived from chitin by deacetylation. Chitosan has been tested as an antioxidant in meat products. Most of these evaluations were performed when chitosan was added as an ingredient. The rate of lipid oxidation in fresh pork sausages was significantly decreased by addition of increasing levels of chitosan (Soultos, Tzikas, Abrahim, Georgantelis, & Ambrosiadis, 2008). Lipid oxidation was lower in emulsion type sausages with chitosan oligomers than in controls, after 3 weeks in aerobic packaging (Jo, Lee, Lee, & Byun, 2001). Refrigerated ground beef patties with added chitosan demonstrated lower lipid oxidation than controls in different modified atmosphere packaging (MAP) systems (Suman et al., 2010).

Chitosans antioxidative action, when applied as a coating, was evaluated in fish. Ojagh, Masoud, Razavi, and Hosseini (2010) and Jeon, Kamil, and Shahidi (2002) showed that chitosan coating was effective in retarding the production of primary lipid oxidation products in refrigerated trout and herring fillets. Jeon et al. (2002) showed that TBARS values in chitosan coated herring and cod samples were lower than in uncoated samples throughout the storage. Similar results were obtained by Mohan, Ravishankar, Lalitha, and Srinivasa Gopal (2012) for Indian oil sardine and Sathivel, Liu, Huang, and Prinyawiwatkul (2007) for pink salmon.

To promote the antioxidative effect of chitosan, plant essential oils can be added to the chitosan film (Georgantelis, Blekas, Katikou, Ambrosiadis, & Fletouris, 2007; Siripatrawan & Noipha, 2012; Xiufang & Baohua, 2008). It was shown that chitosan films enriched with oregano essential oil are an excellent system for controlled release of active compounds (Chi, 2004). Oregano essential oil and extracts, (containing two important active compounds thymol and carvacrol) were shown to have antioxidative effects in different mediums (Almeida-Doria & Regitano-D'arce, 2000; Pitaro, Fiorani, & Jorge, 2012; Ruberto & Baratta, 2000).

The objective of this study was to examine the potential antioxidative effect of a chitosan coating with added oil of oregano on dry fermented sausage, "Petrovská klobása" (label of geographical origin). Examination

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was based on fatty acid profile, measurement of aldehyde contents and sensory analysis of odor and flavor after 2 and 7 months of storage.

2. Material and methods

2.1. Material

2.1.1. Coating preparation

Commercial highly viscous chitosan from crab shells, was purchased from Sigma-Aldrich Chemical (USA). Oregano essential oil was purchased from Aromara (Croatia), glacial acetic acid and Tween 20 were obtained from Superlab (Serbia).

Chitosan coating was prepared by dissolving chitosan powder in 1% acetic acid to reach a chitosan mass per volume ratio of 4 $kg \cdot m^{-3}$. The solution was stirred overnight with a magnetic stirrer in order to dissolve chitosan. Oregano essential oil (0.2% volume concentration) and wetting agent Tween 20 (0.1% volume concentration) were added to the solution.

2.1.2. Sausage preparation

Sausages were made of lean pork meat and fat in the ratio of 80:20. 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. No starter was added, thus fermentation was spontaneous. Sausages were smoked at 5 °C–10 °C and RH = 75%–85%. Conditions depended on outdoor atmospheric conditions. After smoking, sausages were processed in a drying room to reach 35.0% moisture content. In the drying phase, the controlled conditions were 8 °C–10 °C, RH = 90%–75%. The procedure is described in details in Krkić et al. (2012).

2.1.3. Experimental design

After drying was complete, one-half of the sausages were coated with three layers of a coating solution using a sponge brush (assigned as coated sausages). Every layer was left to dry over night before the next layer was applied. The other half of the sausages were left uncoated (assigned as control sausage). After coating, all sausages were stored in chamber with controlled temperature and relative humidity of 15 °C and 75% for seven months. Fatty acid profile and aldehyde content were determined before coating and after two and seven months of storage. All determinations were made on three samples from each group (coated and control) in duplicate.

2.2. Methods

2.2.1. Fatty acid profile determination

The method of Folch, Lees, and Stanley (1957) was used for the extraction of lipids from sausages. The fatty acid composition was determined by gas chromatography. For the preparation of fatty acid methyl esters, KOH/methanol was used. A Perkin-Elmer Varian, series 1400 gas chromatograph fitted with a packed column (3 m × 3.0 mm, a stationary phase GP 10% SPTM-2330 on inert carrier 100/120 Chromosorb WAW) and flame ionization detection was used. The temperature of both the injection port and the detector was 250 °C. The carrier gas was nitrogen, with the flow rate of 20 mL/min. The sample volume was 2.0 μL. Identification of the fatty acid methyl esters was by comparison of the retention times of the peaks in the sample with those of standard pure compounds (Sigma-Aldrich Chemical, USA). Fatty acid methyl esters were quantified as percentage of total methyl esters.

2.2.2. Aldehyde determination

Static headspace gas chromatographic (SHS-GC) analyses were performed on an Agilent 7890A GC System (Agilent Technologies, USA) equipped with a capillary split/splitless inlet, total electronic pneumatic control of gas flow, headspace autosampler and FID. Static headspace (SHS) sampling was performed with the headspace sampler, CombiPAL System (CTC Analytics, Zwingen, Switzerland). A 2.5 mL HS syringe for CombiPAL was used for injection of 2.0 mL of vapor phase from the 10 mL headspace vials. Chromatographic conditions and aldehyde standard preparations were according to Mandić, Sedej, Sakač, and Mišan (2012). Homogenized samples were accurately weighed (2.00 g) into 10 mL screwcapped headspace vials.

2.2.3. Sensory analysis of odor and flavor

A panel of 16 assessors, 9 female and 7 male employees of the Faculty of Technology and Institute for Food Technology, were selected. The assessors had previous experience with sensory analysis, all having already been members of meat product evaluation panels. All were regular consumers of dry fermented sausages and they were all trained according to ISO 8586-1 (1993). The samples were presented in the laboratory for sensory analyses (ISO 8589, 2007), where the assessors were asked to evaluate the sensory characteristics.

Odor and flavor were assessed on a 100 mm unstructured line marked optimum (right end) and unacceptable (left end) (ISO 4121, 2003; Tomović et al., 2013).

2.2.4. Statistical analysis

Statistical analysis was carried out using STATISTICA 8.0 (StatSoft, Inc., 2008). All data were presented as mean values with their standard deviations (mean ± SD). Variance analysis (ANOVA) was performed, with a confidence interval of 95% ($p<0.05$). Means were compared by Duncan's multiple range test.

3. Results and discussion

Table 1 shows the fatty acid profile of the sausages after drying and after 2 and 7 months of storage. After two months storage, there was no change in $\sum$SFA and $\sum$UFA in both coated and control sausages. Decreases were observed in $\sum$PUFA, which have greater susceptibility to oxidation. This decrease was more pronounced in the coated sausages, indicating a higher oxidation.

After seven months storage no significant difference ($p>0.05$) in fatty acid profiles was found between coated and control sausages (Table 1). At this point, values of $\sum$UFA and $\sum$PUFA and ratios of UFA/SFA and PUFA/SFA were slightly higher in the coated sausage, but these values were not significantly different for both groups of sausage ($p>0.05$).

Similarly, Rubio, Martínez, García-Cachán, Rovira, and Jaime (2008) reported that different packaging systems (vacuum and 20% CO_2/80% N_2) and the storage time (210 days) did not effect the fatty acid composition of Spanish dry fermented sausage, salchichón. Valencia, Ansorena, and Astiasarán (2006) found no differences among the fatty acids in dry fermented sausages at the end of drying with those obtained after 30 and 90 days of storage under vacuum.

In Table 2 aldehyde contents after drying, 2 and 7 months of storage are shown. Aldehydes are important lipid-derived volatiles as they have low odor threshold values. They can produce a wide range of flavors and odors. The low perception thresholds make them important to the aroma, even at trace amounts (Sun, Zhao, Zhao, Zhao, & Yang, 2010). A typical oxidation indicator derived from linolenic acid (C18:3) degradation is propanal (Pignoli, Bou, Rodriguez-Estrada, & Decker, 2009). In this experiment, propanal was the most abundant aldehyde produced during storage, for both coated and control sausages. This is in accordance with the results of Josquin, Linssen, and Houben (2012). Hexanal is a secondary lipid oxidation product, derived from linoleic acid (C18:2) which significantly affects the oxidized flavor of all meats and in particular pork (Fernando, Berg, & Grün, 2003; Pignoli et al., 2009). In this study, hexanal which imparts a green grass odor was not the most abundant aldehyde. This result is contrary with those observed by some authors (Olivares, Navarro, & Flores, 2009, Sun et al., 2010; Valencia et al., 2006). It is possible that the low content of hexanal is specific for the aroma of Petrovská klobása. Contents of propanal, pentanal and hexanal increased during storage ($p<0.05$), unlike heptanal and