AN APPLICATION OF BLEND OF NATURAL ANTIOXIDANTS IN DRY-FERMENTED SAUSAGES

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Background

One of the main reasons for limitation of the quality and acceptability of dry-fermented meat products is the lipid peroxidation. This process causes discoloration, appearance of unusual odour and taste and generation of potential toxic compounds (Morrissey et al., 1994; Gray et al., 1996). The investigators examine this problem a long time ago. In the most contributed form this process has not actuality for scientists, but in the poorly developed form - warmed over flavor (WOF) it exists as a problem for some foods, including meat products (Spainer et al., 1992). The "Lukanka" type dry-fermented sausages, traditional for Bulgaria and Balkan's region, are an interesting model for study of this subject, because they are made by mincing, grinding, and mixing of meat raw materials with salt, additives and spices, and after that filling in casings, fermentation (known as maturation), and drying. The sausages are exhibited on significant impact of prooxidant factors during their processing. As a result of those three types of lipid derivatives are formed: primary products - hydroperoxides, secondary products expressed as free malondialdehyde (free MDA) and oxidized cholesterols. The protection of the sausages against lipid peroxidation must start from the beginning of meat storage and to continue during sausage processing. The oxygen intake, exposure to light, and the high temperature must be restricted during the sausage maturation (Lai et al. 1995). The manners for restriction of lipid and pigment peroxidation showed above are not useful or are insufficient effectively, during processing of "Lukanka" type dry-fermented sausages. This is a reason to add different antioxidants and synergists to the minced meat. Those types of blends have not universal application, because their effect depends on the proximate composition of oxidized substrate (Raharjo et al., 1993; Trout and Dale, 1990). The effective stabilization of the lipids in dry-fermented sausages can be realized only after determination of the impact of endogen pro- and antioxidant factors.

Objectives

The objective of this study is to estimate the efficiency of previously developed blend of natural antioxidants (Dragoev et al., 2004) on the progress of peroxidation processes in "Lukanka" type dry-fermented sausages.

Materials and methods

The model system of "Lukanka" was prepared from: 1) chilled beef type CL 95 stored 72 h *post mortem* at 0 – 4 ^{O}C and frozen to minus 5^{O}C 6 h before grinding and 2) chilled pork sort 50/50, stored 72 h *post mortem* at 0 – 4 ^{O}C and frozen to minus 10^{O}C 6 h before cutting, and 3) frozen bacon to minus 10^{O}C, stored 24 h *post mortem*, before using. The experiments were carried out with "Monastiry's lukanka" which is largely covered representative of dry-fermented sausages in Bulgaria, and is distinguished with comparatively high fat content. The last one is a precondition for the most expressive changes of lipid fraction in comparison with other assortments lukanka. The recipe of the "Monastiry's lukanka" control sample is: beef thigh or shoulder blade - 55 kg, pork sort 50/50 semi fatty meat - 25 kg , bacon from the back - 20 kg, salt – 2.200 kg, potassium nitrate – 0.040 kg, black pepper – 0.300 kg, cumin – 0.200 kg, and red pepper – 0.200 kg.
Some parallel samples were processed: Control sample – without antioxidants; The first experimental sample with addition of 0.124 % or 1.24 g/kg blend of natural antioxidants № 1 (liquid form), which contains 48.39 % LRSE 2 – liquid rosemary extract purchased from "Aromena" Ltd - Sofia (Bulgaria). This preparation represents a 28 – 30 % non distillated alcohol extract with flavonoide concentration 2.12 – 2.64 %. The rosemary extract was combined in the blend with 35.48 % chemical pure routine bought from E. Merck (Darmstadt, Germany) and 16.13 % pure sodium erythorbate under trade mark "Eribate", provided from F.I.A. Food Ingredients Anthes GmbH (Teising, Germany); The second experimental sample with addition of 0.112 % or 1.12 g/kg blend of natural antioxidants № 2 (dry form), which contents 3.58 % DRSK – powder rosemary concentrate, containing approximately 42 % flavonoides, mixed with 78.57 % DKFK - dry powder extract of flower bud of the Japanese acacia (*Sophora japonica*), produced in the Department of

Biotechnology in the University of Food Technology – Plovdiv, Bulgaria. The extract contents 53.33 % quercetine - agucone of the natural glycoside routine and 17.85 % pure sodium erythorbate under trade mark "Eribate", provided from F.I.A. Food Ingredients Anthes GmbH (Teising, Germany).
For analysis were purchased following reagents: 2-thiobarbituric acid, distillated pure chloroform and methanol - from Sigma Chemical Company Ltd. (St. Louis, USA, Deisenhofen, Germany); potassium iodide, sodium thiosulfate and silver iodide were purchased by Fluka Chemie AG (Buchs, Swaziland). All rest chemicals and dissolvents were AR and GPL grade and were supplied by Aldrich Chemical Co (Steinheim, Germany).
The samples of "Monastiry's lukanka" were processed using traditional Bulgarian technology expresses in following. The beef was minced by grinder through bars with diameter of holes 5 mm. The beef, pork and bacon were cut to particle size 3 - 4 mm. The salt and spices were added during cutting. The casings were filled with mass using vacuum filler. The pieces of "Lukanka" were formed by clips automat. The separated pieces were dried 2 days at temperature 8 – 9^{O}C and humidity 95 – 90 %. The drying process continues next 18 days at temperature 9 – 11^{O}C and humidity 85 – 75 %. The dry finish product was packaged and labelled.
The total lipids were extracted from the minced sausages by the Bligh and Dyer method (1959).
The oxidative stability of the extracted lipids was determined by Rancimat method (Ranfft et al., 1988), using apparatus Metrohm 679 Rancimat (Metrohm AG, CH – 9100, Switzerland).
The hydroperoxide concentration was determined when the peroxide value (POV) was established using standard iodometric method, presented as meqvO_2.kg^{-1} lipids (AOAC, 1990).
The content of secondary derivatives express by free malondialdehyde was determined using as indicator TBARS (Thiobarbituric acid reactive substances). The water-acid extraction method was used (Schmedes and Hølmer, 1989). TBARS were presented as mg MDA.kg^{-1} sausage.
Microbiological status. The characterization of the microbiological status of the sausages was made by determination of total number of aerobic micro organisms, and oxidase reducing bacteria (ORB). Those indexes were established by standard cultural methods (Boshkova, 2000).
The results were processed statistically by standard methods using computer program Excel.

Results and discussion

Oxidative stability of lipids. The addition of blend of natural antioxidants (in two examined variants) stabilizes sausage lipids (Fig. 1). On the 30th d the induction period of the lipids of experimental samples is approximately 5 times longer then those of the control sample. Two forms of blend of natural antioxidants identically stabilize the extracted lipids.

Figure 1. Oxidative stability of lipids, extracted from "Monastery's lukanka" processes from 1 d stared at 4^{o}C raw materials when is added the blend of natural antioxidants

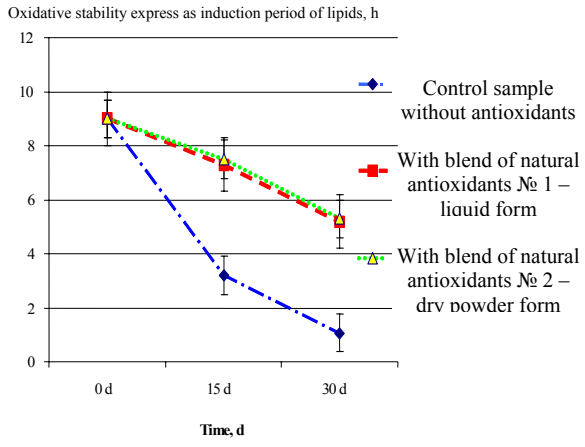
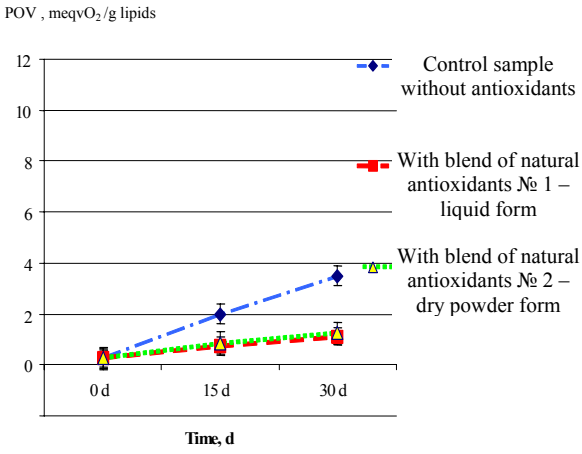


Figure 2. Peroxide value (POV) of lipids, extracted from "Monastery's lukanka" processes from 1 d stared at 4^{o}C raw materials when is added the blend of natural antioxidants

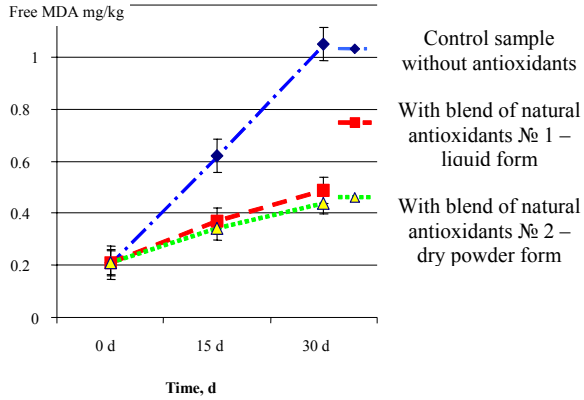


Peroxide value (POV). The addition of blend of natural antioxidants in the mentioned above concentrations in a significant degree suppress accumulation of the primary derivatives of lipid peroxidation (Fig. 2). On the 15th day of drying the hydroperoxide levels in the experimental samples are approximately two times lower than those in control sample, and on the 30th day three times lower. During all examined periods of time two

experimental forms of blend of natural antioxidants do not show statistically different results ($p > 0.05$) regarding hydroperoxide levels.

Thiobarbituric acid reactive substances (TBARS). The addition of blend of natural antioxidants retards dissemination of the chain and transformation of hydroperoxides in secondary derivatives of lipid peroxidation during maturation of the sausages (Fig. 3). The levels of free MDA are almost double time reduced. The accumulation of secondary derivatives of lipid peroxidation delays identically from the two forms of examined blend of antioxidants. The levels of free MDA on 15th d of maturation in the experimental samples are around 40 % lower in comparison with control sample. The level of free MDA in sample № 1 – liquid form of blend on 30th d is lower approximately with 52 % in comparison with the control sample, but in sample № 2 – dry form of blend it is lower around 57 %. Two experimental forms of the blend of natural antioxidants do not show statistically significant differences ($p > 0,05$) regarding TBARS levels, but are statistically significant lower ($p < 0,05$) in comparison with control sample.

Figure 3. TBARS of lipids, extracted from "Monastery's lukanka" processes from 1 d stared at 4°C raw materials when is added the blend of natural antioxidants



The results obtained by us are confirmed by data reported from other investigators (Fernandez and Rodriguez, 1991; Johansson et al., 1994; Chiretti et al., 1997; Chizolini, 1998; Novelli et al., 1998; Zanardi et al., 1998) which are examined typical for Spain, Italy or France dry-fermented sausages and hams. In this case, the indexes of lipid oxidation are within the confines of POV around 2 - 4 meqvO2/кg lipids and TBARS around 0.1 – 0.3 mg MDA/kg.

Microbiological analysis. The tendency of decreasing of the total number of aerobic microorganisms, both in control samples, as well as in two experimental samples were determined (Fig. 4). The addition of blend of natural antioxidants contributes to

Figure 4. Changes of total number of aerobic micro organisms in "Monostery's lukanka" at addition of the blend of natural antioxidants

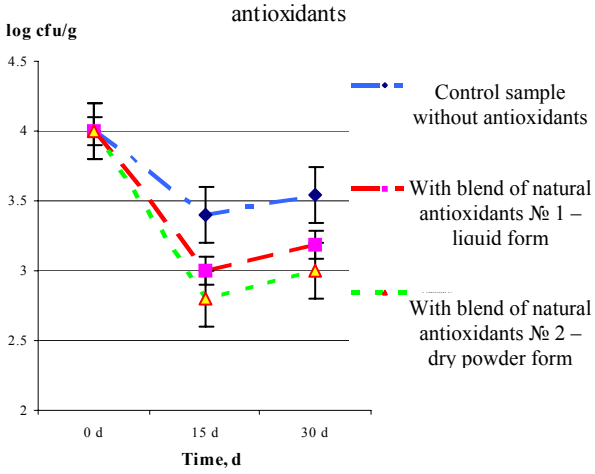
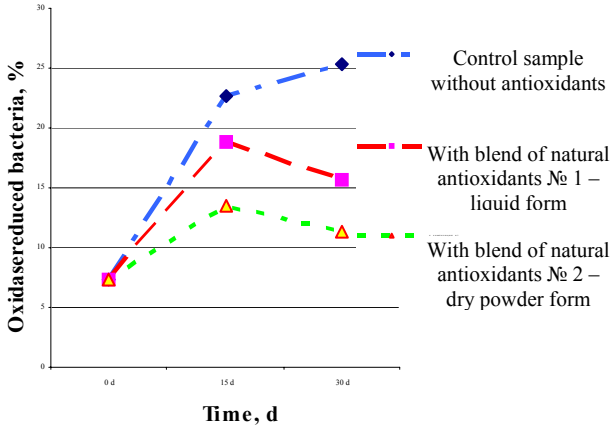


Figure 5. Percentage of ORB in samples "Monastery's lukanka" when is addied the blend of natural antioxidants



statistically significant ($p < 0.05$) decrease of the total number of aerobic microorganisms in "Monastiry's lukanka". The differences of the total number of aerobic micro organisms between two samples with addition of the blend of natural antioxidants are not statistically significant ($p > 0.05$). The percentage of oxidase reducing bacteria increases during maturation of the sausages (Fig.5). In the control sample (30 d) their percentage is statistically significant higher ($p < 0.05$) in comparison with those determined in two experimental samples. The results obtained show, that when the blend of natural antioxidants is added the percentage of oxidase reducing bacteria significantly decreases and the oxidative processes also delay. This increasing is the most significant (around 10 %) on the 15th d. One reason for the considerable decreasing of the hydroperoxides (Fig. 3) in the experimental samples in comparison with the control one is very likely due to decreasing of the percentage of oxidase reducing bacteria.

Conclusions

The two experimental samples of the blend of natural antioxidants stabilized the sausage's lipids and reduced the levels of hydroperoxides and TBARS. The examine blend of natural antioxidants, independently from its forms (liquid or powder), has not clearly bactericide action, because weakly influences on the changes of total number of aerobic micro organisms. The addition of the blend of natural antioxidants mainly affects on the percentage of oxidase reducing bacteria.

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