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STABILIZATION OF DRY-FERMENTED "LUKANKA"TYPE SAUSAGES WITH NATURAL ANTIOXIDANTS BLEND

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Background

One of the main reasons for the quality deterioration of the dry-fermented sausages is the lipid peroxidation. This process causes discoloration, appearance of unusual odour and taste and formation of potential toxic compounds (Morrissey et al., 1994; Gray et al., 1996). At less extent the disadvantage called "Worm over flavour" (WOF) exists as a problem in some types of meat foods (Spainer et al., 1992).

The dry-fermented "Lukanka" type sausages, traditional for Bulgaria, appear to be an attractive model of investigation, as the meat raw materials are minced and mixed with grinded salt, additives and spices, and after that filled in casings, fermented (matured), and dried. During their processing the sausages are exhibited to significant impact of prooxidant factors. As a result three types of lipid derivatives are formed: primary products – lipid hydroperoxides, secondary products - expressed as free malondialdehyde (free MDA) and oxidized cholesterols.

The stabilization of the "Lukanka" type sausages against lipid peroxidation depends on the technological treatment and meat storage. During the "Lukanka" production the abovementioned methods for lipid peroxidation limitation are either inapplicable or insufficiently effective. This is a reason different blend of antioxidants and synergists to be added to the minced meat. These types of blends have not universal application, because their effect depends on the composition of oxidized substrate (Raharjo et al., 1993). The effective stabilization of the lipids in dry-fermented "Lukanka" type sausages can be realized only after determination of the impact of endogen pro- and antioxidant factors.

Objectives

The objective of this study is stabilization of the lipid peroxidation occurring in dry-fermented "Lukanka" type sausages, produced from 3 months stored at -18^{o}C frozen meat raw materials, by the addition of optimized composition of natural antioxidants (Dragoev et al., 2004, Dragoev and Balev, 2004) in two forms – liquid and dry.

Materials and Methods

The model system of "Lukanka" was prepared from frozen meat raw materials: beef type CL 95; pork sort 50/50 and bacon, stored 3 months *post mortem* at -18^{o}C.

The experiments were carried out with "Monastery'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 presented in Table 1.

For the aim of the experiments the following antioxidants were supplied:

LRSE 1 – liquid rosemary extract, supplied by "Aromena" Ltd. (Sofia, Bulgaria). This extract is in accordance with the indicators of the TS (Technological Specification) No 3267-2000 – 55-60 % undistilled alcohol extract, which is a liquid with hard phase, yellow-brown colour and a characteristic rosemary odour. The dry content is 3.00 – 3.10 %. Flavonoides content is 1.15 – 1.30 %. Refraction coefficient Π_D^{20} is 1.3605 and its relative density d^{20}_{20} is 0.7651.

DRSK – dry powder rosemary concentrate, supplied by "Aromena" Ltd. (Sofia, Bulgaria). This concentrate is in accordance with the indicators of the TS (Technological Specification) No 3267-2000 and it is a powder with yellow-brown colour and slightly expressed rosemary characteristic odour. Flavonoides content is about 42 %, and the acid value – 67.87.

RT – chemically pure rutine, purchased from E. Merck (Darmstadt, Germany).

DKFK – dry extract of Japanese acacia (*Sophora japonica*) flower bud**,** produced in the Department of Biotechnology in the University of Food Technologies – Plovdiv, Bulgaria. The dry finely grinded mass is hydrolysed 8 hours at 60^{o}C and pH 4.5. The obtained hydrolyse product is triple extracted with 80 % ethanol and dried (Valkova and Bahcevanska, 2003). The extract contains 53.33 % quercetine – aglicon of the natural glycoside rutine.

SE – sodium erythrobate, supplied by F.I.A. Food Ingredients Anthes GmbH (Teising, Germany).

For the analysis the following reagents were purchased: 2-thiobarbituric acid, distilled pure chloroform and methanol - from Sigma Chemical Company Ltd. (St. Louis, USA, Deisenhofen, Germany); potassium iodide, sodium thiosulfate and silver iodide were supplied by Fluka Chemie AG (Buchs, Swaziland). All the rest of chemicals and solvents were AR and GPL grade and were supplied by Aldrich Chemical Co (Steinheim, Germany).

Parallel samples were produced as following:

Control sample – without antioxidants.

First experimental sample – with addition of 0.124 % (1.24 g/kg) natural antioxidants composition No 1 (liquid form), containing 48.39 % LRSE 2 – liquid rosemary extract, which is 28 - 30 % undistilled alcohol extract with flavanoides content of 2.12 – 2.64 %, combined with 35.48 % rutine and 16.13 % sodium erythrobate.

Second experimental sample – with addition of 0.112 % (1.12 g/kg) natural antioxidants composition No 2 (dry form), containing 3.58 % DRSK – dry powder rosemary concentrate, containing about 42 % flavonoides, combined with 78.57 % DKFK – powder extract of Japanese acacia (*Sophora japonica*) flower bud, containing 53.33 % quercetine – aglicon of rutine and 17.85 % sodium erythrobate.

The samples of "Monastery's lukanka" were processed by use of traditional Bulgarian technology.

The lipids of the samples were extracted by the Bligh and Dyer method (1959), according the recommendations of Smith et al. (1990).

The oxidative stability of the extracted lipids was determined by Rancimat method (Ranfft et al., 1988), based on conductometric measuring of the lipid peroxidation volatile secondary products. The analyses were carried out on the Metrohm 679 Rancimat apparatus (Metrohm AG, CH – 9100, Switzerland).

The hydroperoxide concentration was determined by measuring the peroxide value (POV), expressed as meqvO_2.kg^{-1} lipids (AOAC, 1990).

Lipid peroxidation secondary products content, expressed as free malondialdehyde (MDA) was estimated by TBARS indicator according the water-acid extraction method (Schmedies and Holmer, 1989). TBARS were presented as MDA mg.kg^{-1} product.

Microbiological analyses. The total number of micro organisms and the share of oxidase reducing bacteria were estimated according standard methods (Boshkova, 2000).

Statistical analyses. The data was processed using the Microsoft Excel, Version 5 software. The ANOVA was made. The statistical significant differences were determined by Fischer's test.

Results and Discussion

The addition of natural antioxidants blend (in both examined forms) partially stabilizes the lipids when frozen meat materials are used. Higher and statistically significant differences ($p < 0.05$) are estimated only in the first 15 – 20 d of the maturing and drying of the product. In the final product – at 30 d, all the examined samples show induction period close to 0 h (Fig. 1). The two forms of the antioxidants composition stabilize almost identically the sausage lipids.

The addition of antioxidant composition at the abovementioned concentrations significantly suppresses the formation of the primary products of lipid peroxidation (Fig. 2), where in the maturing and drying process (15 d) the levels of the formed hydroperoxides reduce with 22 – 25 %. In the final product (30 d) the sample with an addition of liquid form No 1 of the composition shows approximately three times lower levels of hydroperoxides with comparison to the control sample, while the sample with an addition of dry form No 2 of the composition shows about 65 % reduction of the hydroperoxides level with comparison to the control sample.

Irrespectively of the significant detention of the lipid peroxidation in the experimental samples of "Monastery's lukanka", produced from frozen raw materials, the levels of the hydroperoxides are higher than 3 meqvO_2/g lipids. These results show that once the lipid peroxidation process during the meat raw materials storage progresses, it is difficult to get control over it during the further technological treatment of the sausages.

It is estimated that both blend forms do not show statistically different results ($p > 0.05$) concerning the hydroperoxides levels, but they are statistically significantly lower ($p < 0.05$) with comparison to the control sample.

With the examination of the effect of antioxidant blend on limitation of the lipid peroxidation secondary products formation, similar results are obtained (Fig. 3). The addition of the antioxidant blend reduces to great extent the levels of the free malondialdehyde. On the 15 d the levels of the lipid peroxidation secondary products formed are reduced with 20 – 22 %, and in the final product (30 d) – with about 25 %.

The sample with the addition of liquid form No 1 of the blend shows statistically significant ($p < 0.05$) lower levels of malondialdehyde on the 15 d of the drying in relation to the sample with addition of dry form No 2 of the blend, but in the final product in both experimental samples the levels of TBARS are statistically indifferent ($p > 0.05$).

It is estimated that both examined forms of the blend do not show statistically different results ($p > 0.05$) in relation to the TBARS levels, but they are statistically significantly lower ($p < 0.05$) than these of the control sample.

The results show that the total number of aerobic mesophylic microorganisms decrease during the sausage drying. This trend of decrease is kept both in the control samples (produced without antioxidants addition) and experimental samples (with dry and liquid form of antioxidant blend addition) (Fig. 4). The addition of natural antioxidant blend contributes to the slight, but statistically significant decrease ($p < 0.05$) of the total number of microorganisms in "Monastery's lukanka", as both in the processes of maturing and drying, as well as in the final product. Little higher are the results of the samples with addition of the liquid form No 1 of the antioxidant blend, in relation to the dry form No 2, but these differences are statistically insignificant ($p > 0.05$) (Fig. 4). The total number of microorganisms in the samples of filling mass of "Monastery's lukanka", produced from frozen raw materials (0 d) is 3.8×10^4. The addition of antioxidant blend does not effects statistically significantly the change of the total number of mesophylic aerobic microorganisms in the samples, produced from frozen raw materials. This shoes, that the antioxidant blend used has not clear bactericide effect, irrespectively of the form (dry or liquid).

During the process of maturing and drying of the sausages, produced from frozen meat raw materials, the number of the oxidase positive strains increases (Fig. 5) and in the control sample of the final product (30 d) the increased percentage of these microorganisms is statistically significantly greater ($p < 0.05$) in relation to the both experimental samples. The results obtained show, that the addition of antioxidant blend contributes to the significant decrease of the number of oxidase positive strains and detains the oxidative processes in the sausages.

Conclusions

The two experimental samples of the natural antioxidant blend stabilize the sausage's lipids and reduce the levels of hydroperoxides and TBARS. The examined blend of natural antioxidants, irrespectively of its forms (liquid or powder), has not clear bactericide action and slightly effects the changes of total number of aerobic microorganisms. It mainly influences the share of oxidase reducing strains and thus suppresses the initialization of free radicals which cause lipid peroxidation processes.

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Table 1. Raw materials of 100 kg filling mass for “Monastiry’s lukanka” processing

Main and supplementary raw materials	**Mass, kg**
1. Beef leg and shoulder	55.000
2. Semi fat pork	25.000
3. Firm dorsal bacon	20.000
4. Salt	2.200
5. Nitrate potassium	0.040
6. Black pepper	0.300
7. Cumin	0.200
8. Red pepper	0.200
9. Artificial casings “Fibrous” type 55 ∅ mm	70 m

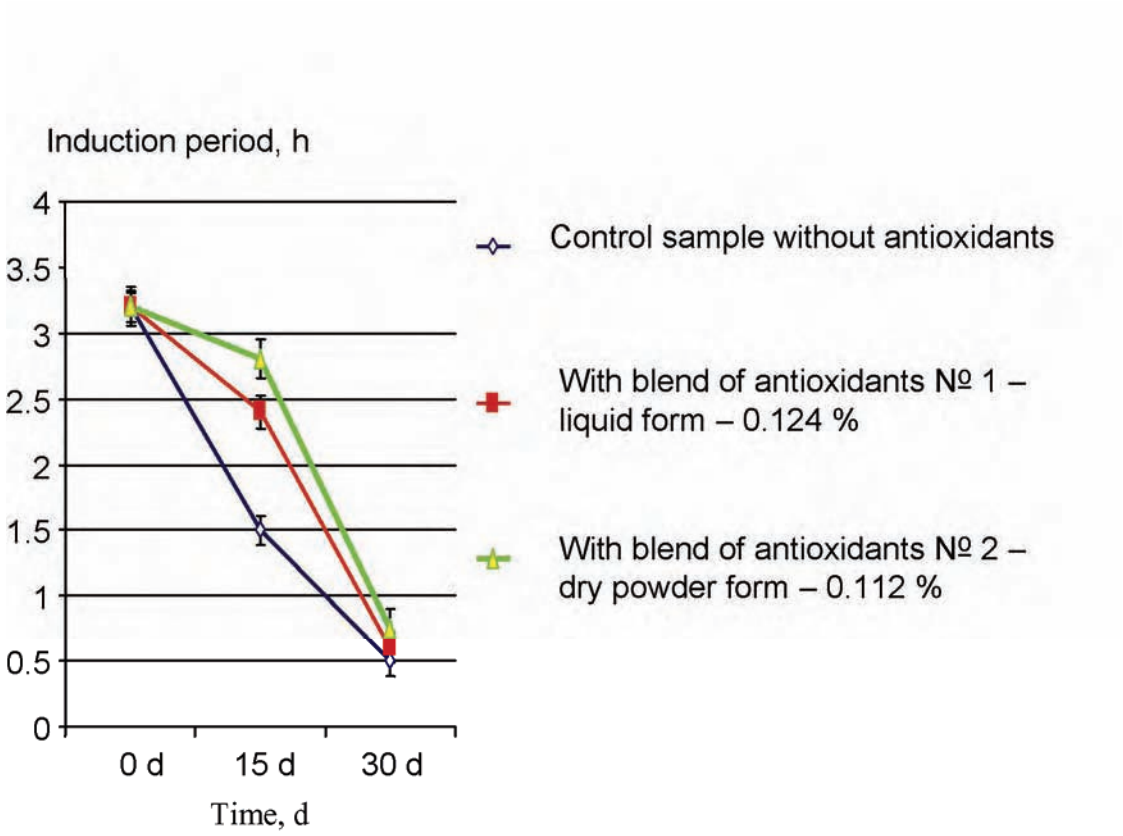

Figure1. Oxidative stability of lipids, extracted from “Monastery’s lukanka” processed from frozen to minus18°C 90 d stored raw materials with addition of antioxidant blend

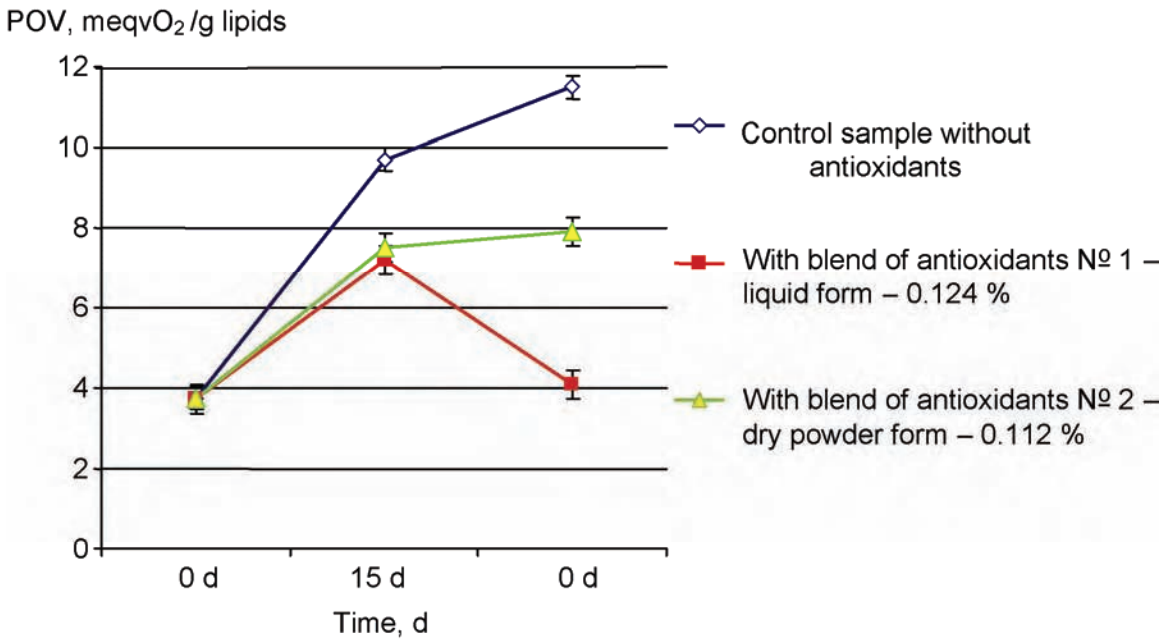

Figure 2. Peroxide value (POV) of lipids, extracted from "Monastery's lukanka" processed from frozen to minus 18°C 90 d stored raw materials with addition of antioxidant blend

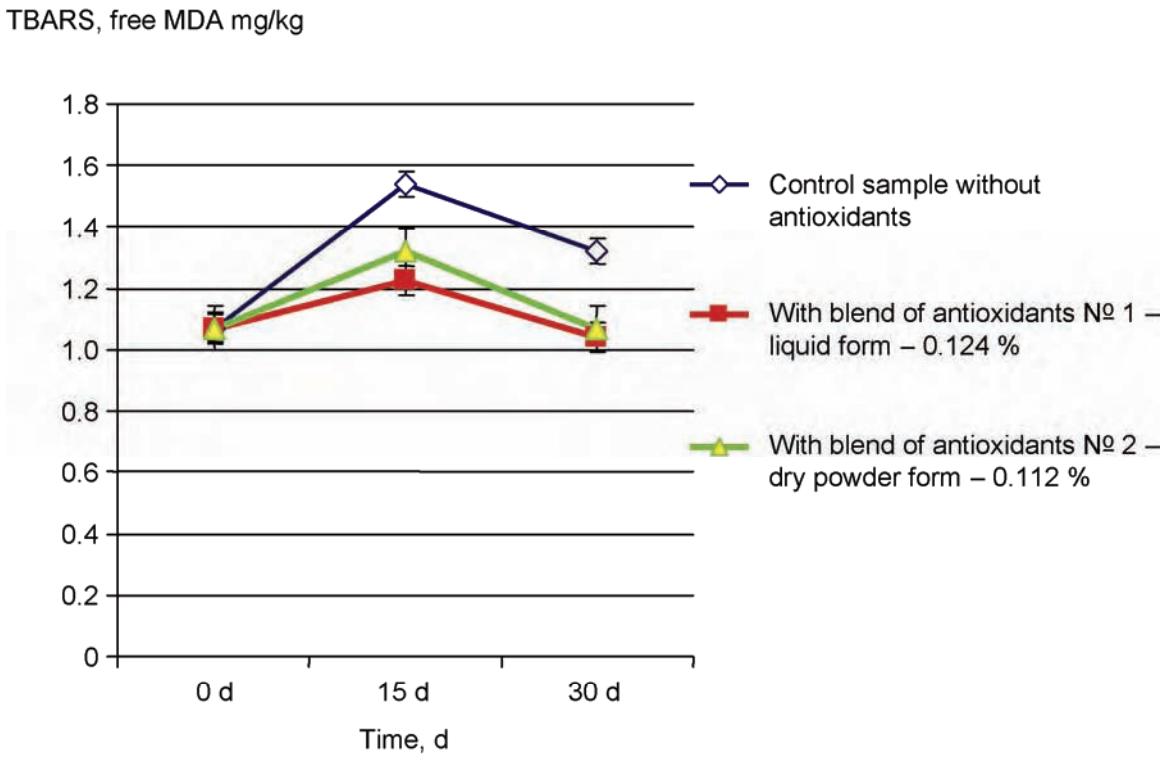

Figure 3. TBARS of lipids, extracted from "Monastery's lukanka" processed from frozen to minus18°C 90 d stored raw materials with addition of antioxidant composition

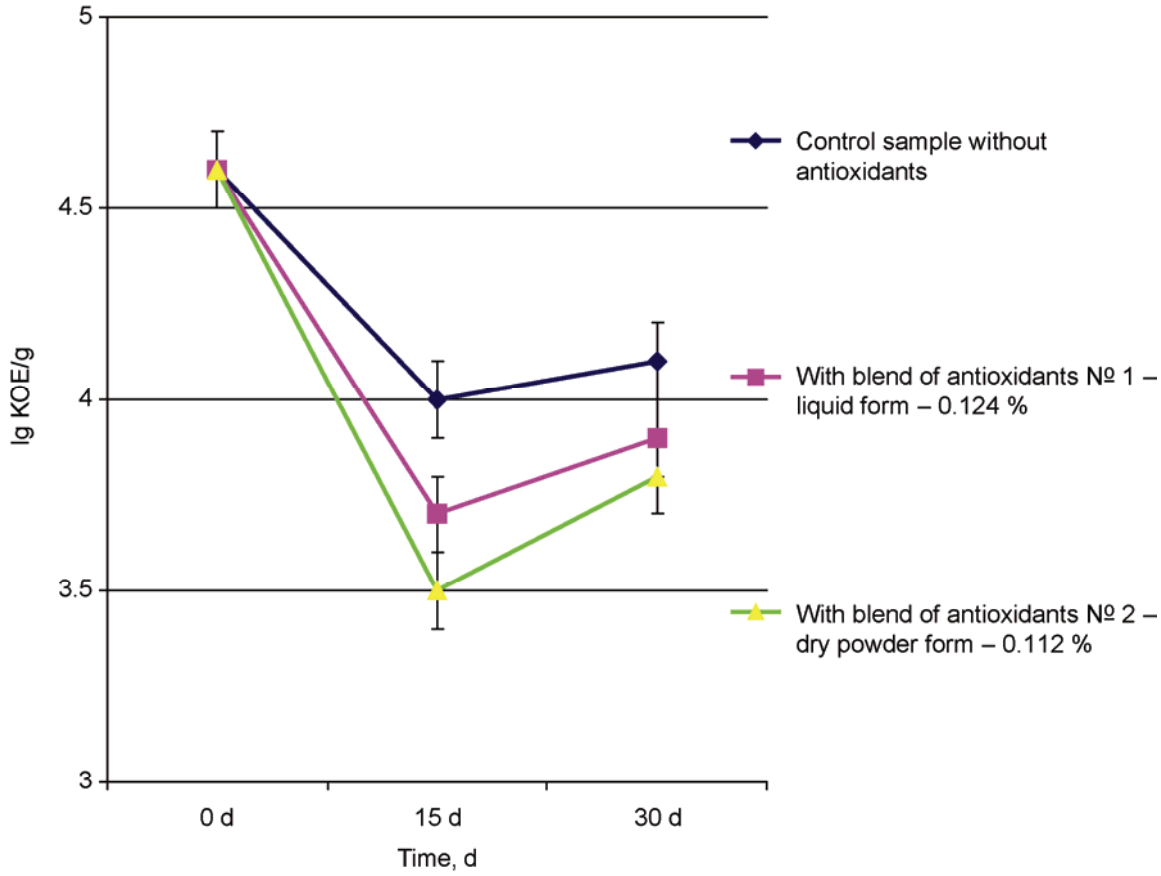

Figure 4. Changes of total number of micro organisms (logKOE/g) in "Monastery's lukanka" processed from frozen 90 d stored raw materials with addition of antioxidant blend

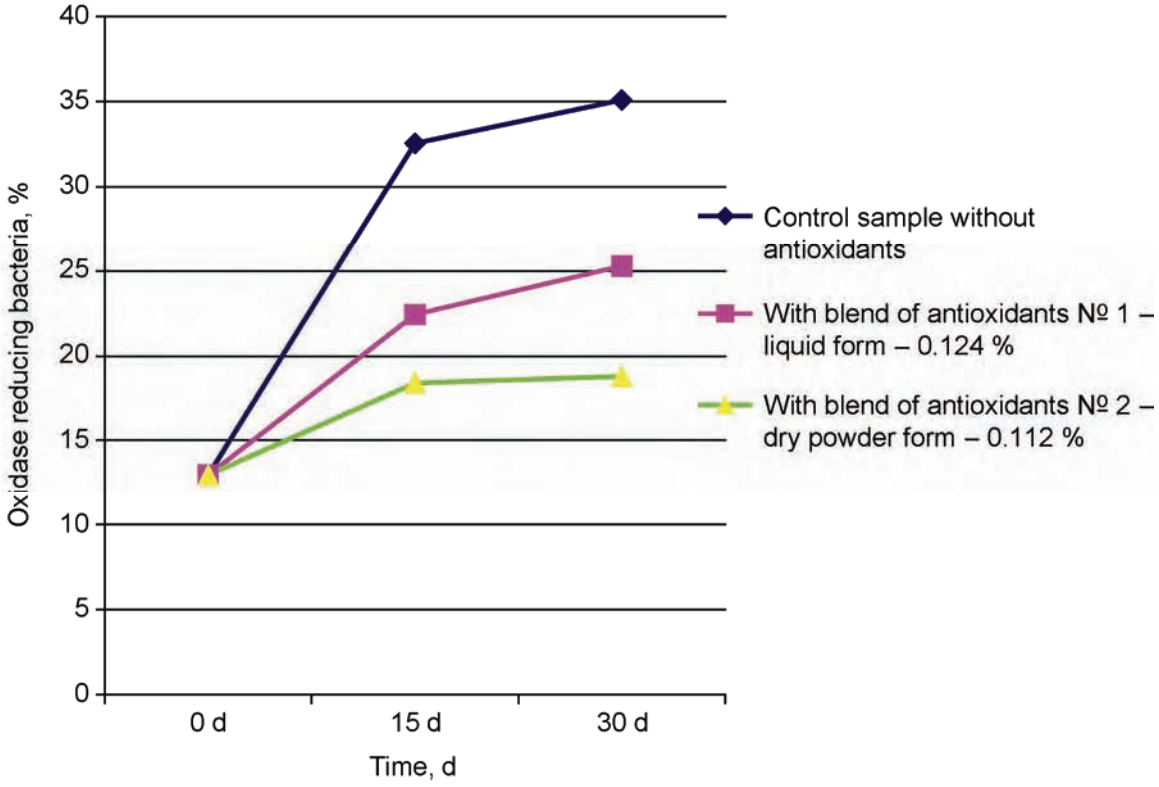

Figure 5. Percentage of ORB in relation to total number of microorganisms in "Monastery's lukanka" processed from frozen to minus 18°C 90 d stored raw materials with addition of antioxidant blend