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EFFECT OF THREE-COMPONENT ANTIOXIDANT BLEND ON OXIDATIVE STABILITY AND NITRITE REDUCTION OF COOKED SAUSAGES

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ABSTRACT

Background. There is a growing interest in the so-called "clean label" meat products and those with a reduced synthetic additives content. The utilization of rose oil by-products is another motive underlying this study. Therefore, the aim of the study is to evaluate the effect of the incorporation of the three-component antioxidant blend on the oxidative stability and antioxidant activity of functional cooked sausages with nitrite reduction.

Materials and methods. The three-component antioxidant blend was formulated after a mathematical optimization according to four target functions and consists of: 0.100 g/kg freeze-dried extract from dry distilled rose petals, 0.091 g/kg dihydroquercetin and 0.100 g/kg sodium L-ascorbate. Six different cooked sausage samples were produced: control – C without antioxidant blend and 100% nitrite, AN100, AN75, AN50, AN25 and AN0 with three-component antioxidant blend and the respective percentage of nitrite. The changes in the residual nitrites, antioxidant activity as well as the hydrolysis and oxidation in lipid and protein fractions were evaluated at days 1, 7 and 14 of chilled storage (0–4°C).

Results. A proportional reduction of the residual nitrites in the final cooked sausages was found. The radical-scavenging activity (DPPH) and iron-reducing activity potential (FRAP) increased with the incorporation of three-component antioxidant blend. Its inclusion promoted inhibition of lipolysis and lipid oxidation in the cooked sausages with up to 50% of a reduction in nitrites. A similar stabilizing effect on the protein oxidation was observed.

Conclusion. The incorporation of three-component antioxidant blend containing X_1 – 0.100 g/kg freeze-dried extract of dried distilled rose (*Rosa damascena* Mill.) petals; X_2 – 0.091 g/kg dihydroquercetin isolate from *Larix sibirica* Ledeb and X_3 – 0.100 g/kg sodium L-ascorbate can successfully stabilize the oxidative processes in functional cooked sausages with a 50% reduction in nitrite content.

Keyword: rose extract, oxidation, meat products, residual nitrites, functional food

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INTRODUCTION

Oxidation processes occurring in cooked meat products reduce both their nutritional value and shelf life (Domínguez et al., 2022). Due to its fat-rich nature and heat treatment, the oxidative stability of cooked meat products is reduced (Domínguez et al., 2019). The end products of lipid peroxidation (malondialdehyde/MDA) and protein oxidation (carbonyls) are the main substances responsible for changing the sensory and quality characteristics of meat products (Estévez, 2011; Sharma et al., 2018). Additionally, those substances pose a potential health hazard (Domínguez et al., 2019; Morsy and Elsabagh, 2021).

The antioxidant and antimicrobial properties of sodium nitrite have been studied substantially through the years (Hernández et al., 2021; Lavado et al., 2021). However, the potential formation of N-nitroso compounds, which are carcinogens, have caused a negative point of view against the usage of nitrites (Choi et al., 2019). The characteristic color of cooked sausages is possible due to the reaction between nitric potential oxide released by the nitrites and the meat red pigment myoglobin. The newly formed nitrosylmyoglobin undergoes thermal denaturation to nitrosohemochromogen to give a nice pink-red color (Shpaizer et al., 2018). The absence of nitrites in the meat matrix leads to oxidation of myoglobin to metmyoglobin and a corresponding change from pink-red to a brownish gray color (Hernández et al., 2021). The reduction of the residual nitrites in the final product through reduction of the added sodium nitrite has led to a decreased stabilizing effect (Wang et al., 2015).

In order to inhibit free radicals' action, antioxidants must be included in the system. Both synthetic and natural antioxidants are capable of scavenging the free radicals, which inhibit oxidative changes in the product. The negative consumer perception to the synthetic antioxidants gives preference to those of natural origin (Sharma et al., 2018). Plant extracts are rich in flavonoids, which exhibit high antioxidant properties (Dinkova et al., 2014). Industrial food processing generates tons of waste products every year. Distilled rose (*Rosa damascena* Mill.) petals are a waste product of the rose oil industry – the ethanol extract contains more than 20 quercetin and kaempferol glycosides (Dragoev et al., 2021). A combination of extracts as blends of antioxidants has been an area of interest in the last 10 years (Sharma et al., 2018). The synergism between the components of the blends allows the use of fewer additives, but at the same time, the antioxidant activity remains the same or even increases (Bulambaeva et al., 2014).

Different blend formulations using freeze-dried extract of dried distilled rose (*Rosa damascena* Mill.) petals – X_1; dihydroquercetin isolate from *Larix sibirica* Ledeb – X_2 and sodium L-ascorbate – X_3 were previously studied (Kolev et al., 2022). The three-component antioxidant blend was formulated by a mathematical optimization according to the target functions. We hypothesize that the incorporation of three-component antioxidant blend in cooked sausages with reduced nitrite content could stabilize the oxidative processes during 14 days of chilled storage (0–4°C).

MATERIALS AND METHODS

For the purpose of the experiment, chilled deboned beef shoulder and pork bacon, at 48 h *post mortem*, were used. The sausages were produced in the Department of Meat and Fish Technology at the University of Food Technology, Plovdiv, Bulgaria according to the technology presented in Figure 1. Three-component antioxidant blend was also produced as follows: X_1 = 0.100 g/kg, X_2 = 0.091 g/kg and X_3 = 0.100 g/kg. All other ingredients are food-grade and were bought locally.

Sample preparation

Sausages at the 1st, 7th and 14th day of chilled storage were homogenized and analyzed immediately according to Esbensen and Wagner (2014).

For the following analyses a double beam UV-VIS spectrophotometer Camspec M550 was used (Camspec Ltd, UK).

Residual nitrites

The spectrophotometric method, described in CSN EN 12014-3:2005, was used to determine the amount of residual nitrite in the final products.

Changes of antioxidant properties

The total antioxidant properties of the sausages was assessed by free radical-scavenging activity (DDPH·

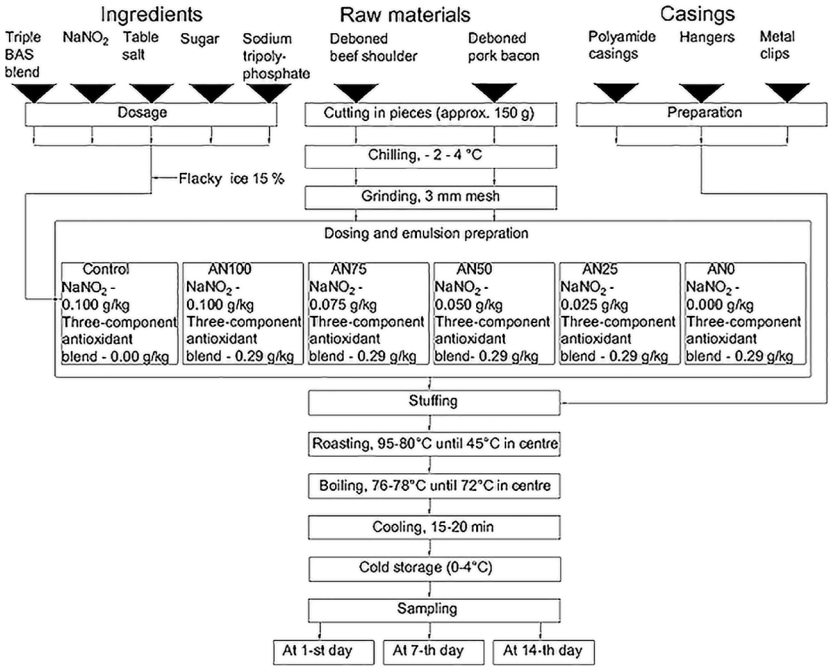

Fig. 1. Experimental design

test) and ferric reducing antioxidant potential (FRAP assay) (Dinkova et al., 2014).

Changes in lipid fraction

The extraction of total lipids was done according to the Bligh and Dyer (1959) method. The hydrolytic changes are expressed by the acid value (AV) and evaluated according to ISO 660:2020. The primary products of lipid peroxidation or peroxide value (POV) are determined by the color reaction between the oxidized Fe^{3+} and SCN (Schmendes and Hølmer, 1989). TBA value expresses the amount of end products of lipid oxidation, in particular, malondialdehyde (MDA). It is evaluated according to the method of Botsoglou et al. (1994).

Changes in protein fraction

Muscle proteins are extracted with phosphate buffer (pH 7.3) as described by Mercier et al. (2004). The hydrolytic changes in protein homogenate are expressed by the content of α-aminoacidic nitrogen, according to the ninhydrin method of Garrido et al. (2012). The degree of protein oxidation was expressed by protein carbonyls accumulation using DNPH assay (Mercier et al., 2004).

Statistical analysis

Two-way ANOVA with replications with a significance level $P \leq 0.05$ ($n = 5$) was executed (Draper and Smith, 1998). Results were presented as means ±SEM; ^{a,b,c} – means in same row differ significantly ($P \leq 0.05$); ^{x,y,z} – means in same column and parameter differ significantly ($P \leq 0.05$).

RESULTS AND DISCUSSION

Residual nitrites

The residual nitrites content was decreased proportionally to the reduction of the added sodium nitrite. The reduction of the added sodium nitrite by 25% (sample AN 75) led to almost double the decrease of the residual nitrite content (Fig. 2). This tendency was persistent throughout the experiment. On the 14th day of chilled storage, an increase in the amount of residual nitrite in the control and AN100 was found. This is most likely a consequence of the ongoing oxidation processes and possible release of nitric oxide (NO) from the heme pigments. At the same time, the residual nitrites content in AN75 and AN50 did not differ significantly ($P > 0.05$) and was stabilized around 6 to 4 mg $NaNO_2$/kg (Fig. 2), respectively.

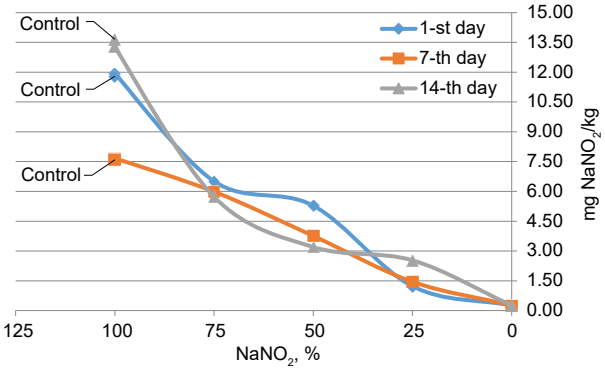

Fig. 2. Residual nitrites of the cooked sausages during the chilled storage

Similar results were obtained by Lavado et al. (2021) for dry-cured loin with reduced nitrite addition. The residual nitrites content of all the studied cooked sausages were below the regulated maximal dose of 15 mg $NaNO_2$/kg or 15 ppm reported by Hernández et al. (2021). Lower amounts of residual nitrites would mean a reduction in nitrosamines, thus increasing the quality and safety of finished products (Shpaizer et al., 2018).

Changes in antioxidant activity

The control sample was characterized by a trending to zero radical-scavenging ability (DPPH•), while experimental samples with the addition of three-component antioxidant blend showed significantly ($P \leq 0.05$) higher values (Table 1). A decreased radical-scavenging ability when decreasing the amount of sodium nitrite was observed, which confirms the previously reported nitrites antioxidant properties (Wang et al., 2015; Lavado et al., 2021). Secondly, a reduction of DPPH values during the storage was observed, probably caused by the ongoing oxidative processes and the consumption of substances with antioxidant properties. Similar observations were stated by Sharma et al. (2018). The observed oxidative changes in lipid fraction (Table 2) confirm the decrease of the antioxidant activity, suggesting an induction period until the 7th day and subsequently rapid increase of the oxidation processes and antioxidant depletion until the 14th day.

The trend in the iron-reducing activity potential (FRAP) of sausages is similar to their radical-scavenging ability (DPPH•). The control marks the lowest values for the whole period of the experiment (Table 1). The relatively similar FRAP in the absence of nitrites (AN0) confirms that their antioxidant potential is against the free radical and non-metal ions (Wang et al., 2015; Lavado et al., 2021). Experimental sausages with a three-component antioxidant blend are characterized by similar FRAP values on the 1st and 7th day which at the end of the experiment decreased by 3 to 5 times. The low iron-reducing potential at the end of the experiment was in agreement with the estimated ongoing oxidative processes (Table 2).

Table 1. Changes in the antioxidant activity of the cooked sausages, during chilled storage, µmol TE/100 g

Sample / Parameter	C	AN100	AN75	AN50	AN25	AN0
			1st day			
DPPH	$41.00^{a,z} \pm 0.69$	$259.01^{f,y} \pm 2.51$	$224.64^{e,y} \pm 2.92$	$173.33^{d,y} \pm 3.01$	$162.93^{c,y} \pm 1.38$	$145.99^{b,y} \pm 2.22$
FRAP	$158.28^{a,z} \pm 0.53$	$542.14^{d,y} \pm 1.33$	$537.67^{d,z} \pm 1.71$	$491.14^{b,y} \pm 1.61$	$492.01^{b,y} \pm 1.77$	$507.83^{c,z} \pm 1.11$
			7th day			
DPPH	$31.33^{a,y} \pm 0.61$	$248.92^{e,y} \pm 0.98$	$232.96^{d,y} \pm 1.45$	$160.85^{c,y} \pm 1.65$	$144.45^{b,y} \pm 0.66$	$141.44^{b,y} \pm 1.28$
FRAP	$139.16^{a,y} \pm 0.33$	$542.49^{d,y} \pm 1.57$	$492.62^{c,y} \pm 1.47$	$485.61^{c,y} \pm 0.89$	$490.57^{c,y} \pm 1.90$	$466.42^{b,y} \pm 1.45$
			14th day			
DPPH	$25.33^{a,x} \pm 0.81$	$88.78^{e,x} \pm 0.59$	$65.55^{d,x} \pm 1.39$	$49.17^{c,x} \pm 0.67$	$46.16^{c,x} \pm 0.49$	$38.40^{b,x} \pm 0.77$
FRAP	$17.50^{a,x} \pm 0.71$	$216.65^{f,x} \pm 1.44$	$172.04^{d,x} \pm 1.74$	$188.55^{e,x} \pm 1.89$	$138.82^{c,x} \pm 2.30$	$114.80^{b,x} \pm 1.56$

Table 2. Changes in the lipid fraction of the cooked sausages during the chilled storage

Sample / Parameter	C	AN100	AN75	AN50	AN25	AN0
			1st day			
AV, mg KOH/g	$1.54^{b,z} \pm 0.04$	$0.74^{a,x} \pm 0.04$	$0.70^{a,x} \pm 0.05$	$0.81^{a,x} \pm 0.03$	$0.73^{a,x} \pm 0.04$	$0.74^{a,x,y} \pm 0.07$
POV, meq O_2/kg	$1.36^{a,y} \pm 0.07$	$1.39^{a,y} \pm 0.05$	$1.35^{a,x} \pm 0.06$	$1.43^{a,x} \pm 0.06$	$1.44^{a,x} \pm 0.08$	$1.42^{a,x} \pm 0.06$
TBA, mg MDA/kg	$0.31^{a,x} \pm 0.03$	$0.28^{a,x} \pm 0.02$	$0.29^{a,x} \pm 0.03$	$0.34^{a,x} \pm 0.01$	$0.36^{a,x} \pm 0.07$	$0.30^{a,x} \pm 0.05$
			7th day			
AV, mg KOH/g	$1.28^{b,y} \pm 0.05$	$0.83^{a,x} \pm 0.09$	$0.90^{a,y} \pm 0.07$	$0.92^{a,y} \pm 0.05$	$0.87^{a,y} \pm 0.07$	$0.89^{a,y} \pm 0.09$
POV, meq O_2/kg	$1.30^{a,y} \pm 0.05$	$1.54^{b,z} \pm 0.05$	$1.61^{b,y} \pm 0.04$	$1.67^{b,y} \pm 0.05$	$1.68^{b,y} \pm 0.09$	$1.51^{b,y} \pm 0.06$
TBA mg MDA/kg	$1.03^{c,z} \pm 0.06$	$0.47^{a,b,y} \pm 0.07$	$0.51^{b,y} \pm 0.01$	$0.42^{a,y} \pm 0.01$	$0.40^{a,x} \pm 0.01$	$0.54^{b,y} \pm 0.02$
			14th day			
AV, mg KOH/g	$0.47^{a,x} \pm 0.06$	$0.80^{c,x} \pm 0.05$	$0.80^{c,x,y} \pm 0.08$	$0.82^{c,x} \pm 0.05$	$0.69^{b,x} \pm 0.05$	$0.65^{b,x} \pm 0.05$
POV, meq O_2/kg	$1.09^{a,x} \pm 0.03$	$1.27^{b,x} \pm 0.05$	$1.41^{c,x} \pm 0.05$	$1.44^{c,x} \pm 0.08$	$1.39^{b,c,x} \pm 0.07$	$1.43^{c,x} \pm 0.05$
TBA, mg MDA/kg	$0.77^{c,y} \pm 0.03$	$0.50^{b,y} \pm 0.03$	$0.49^{b,y} \pm 0.02$	$0.45^{a,b,y} \pm 0.04$	$0.42^{a,b,x} \pm 0.05$	$0.38^{a,x} \pm 0.05$

Changes in the lipid fraction

On the 1st and 7th days, the highest degree of lipid hydrolysis was measured in the control sample (Table 2), approx. 1.4–2 times higher than the experimental samples. The levels of free fatty acids were stabilized with the addition of the three-component antioxidant blend (Table 2) during the 14th day of the chilled storage of the sausages, regardless of different concentrations of added sodium nitrite.

Bulambaeva et al. (2014) reported a similar trend in cooked sausages with reduced nitrite content. This phenomenon can be explained by the inhibitory effect of the dihydroquercetin against the free radicals (Vlahova-Vangelova et al., 2014), and the chelate effect of the extract of distilled rose petals (Dragoev et al., 2021) manifested in the conditions of thermally initiated lipolysis during the heat treatment of sausages. Thus, in the control sample free fatty acids appear to be reduced (Table 2). This is at the expense of their interaction with reactive radical species and their inclusion in the auto-oxidative process of lipids (Domínguez et al., 2019).

In all tested sausages, a POV peak was observed on the 7th day of chilled storage (Table 2). The results are in an agreement with the theory, indicating manifestation of an induction period in which the content of primary products of lipid peroxidation increases and reaches its maximum, then tends to decrease (Bulambaeva et al., 2014; Morsy and Elsabagh, 2021). The lower levels of POV in the control on the 14th day could be explained by the fact that after the incubation period the decomposition of hydroperoxides is higher than their accumulation (Domínguez et al., 2019). Due to the absence of the three-component antioxidant blend, the process of degradation of lipid hydroperoxides into low molecular weight secondary derivatives are accelerated.

The primary products of lipid peroxidation decay rapidly, forming low molecular weight derivatives (MDA; Botsoglou et al., 1994), demonstrated by the significant ($P \leq 0.05$) increase in TBA value in these samples (Table 2). During the chilled storage of the sausages, the TBA value of the control sample increased more than twice, with a characteristic peak at the 7th day, corresponding to the induction period of the lipid peroxidation process observed in the POV (Balev et al., 2019; Morsy and Elsabagh, 2021; Vlahova-Vangelova et al., 2014). The evaluated stabilization of MDA levels in AN100, AN75, AN50, AN25 between the 7th and 14th day confirms the antioxidant potential of the blend (Table 1). The decrease of the TBA value in AN0 and C at the end of the experiment

suggests that the secondary products of the lipid peroxidation are continuing the oxidation processes in the protein fraction (Domínguez et al., 2022; Estévez, 2011).

Changes in protein fraction

In Table 3, the data for the changes in free amino groups (α-aminoacidic nitrogen) in sausages during the chilled storage is presented. Between the 1st and 7th day of the chilled storage, ongoing hydrolyze and an increase ($P \leq 0.05$) of the free amino groups was observed in all the samples. The values thus established are relatively low and with little fluctuation. On the 14th day, no significant differences ($P > 0.05$) were found between all the sausages examined, but the values of α-aminoacidic nitrogen decreased compared to the previous days. The obtained results are in agreement with the data from previous studies with other model systems of meat products (Bulambaeva et al., 2014; Vlahova-Vangelova et al., 2014). It can be concluded that the nitrite reduction and the incorporation of the three-component antioxidant blend in a model system of cooked sausage does not significantly affect ($P > 0.05$) the dynamics of changes in α-aminoacidic nitrogen.

The levels of carbonyl compounds in all tested samples throughout the experiment are very low (Table 3), although the differences between the mean values are significant ($P \leq 0.05$). These results are in contrast to those reported by Estévez (2011) of approximately 3–5 nmol DNPH/mg protein but are in agreement with data reported by Vlahova-Vangelova et al. (2014) and Balev et al. (2019). The amount of protein carbonyl demonstrates that the functional cooked sausages with the addition of a three-component antioxidant blend didn't undergo strong processes of protein oxidation during 14 days of chilled storage. On the 14th day, the weakest oxidative changes were found in the protein fraction of AN100, AN75 (Table 3). The most significant protein oxidation was observed in sausages without incorporated antioxidants and 100% nitrite content (control – C) and in the experimental samples AN0, AN25. Since nitrites exhibit weak antioxidant properties (Wang et al., 2015), their absence in the AN0 reduces the possibility of inhibiting the pro-oxidant action of salt in the filling mass of sausages and thus contributes to more pronounced protein oxidation (Hernández et al., 2021).

The three-component antioxidant blend was able to inhibit the accumulation of protein carbonyls in the

Table 3. Changes in protein fraction of the cooked sausages during the chilled storage

Sample / Parameter	C	AN100	AN75	AN50	AN25	AN0
1st day						
α-aminoacidic nitrogen, mg Leu/g	$120.31^{b,y} \pm 0.63$	$130.03^{c,y} \pm 0.56$	$141.66^{d,z} \pm 0.42$	$119.20^{b,y} \pm 0.77$	$132.08^{c,z} \pm 0.55$	$113.33^{a,y} \pm 0.58$
Protein carbonyls, nmol DNPH/mg protein	$0.180^{c,x} \pm 0.005$	$0.111^{a,x} \pm 0.002$	$0.118^{b,x} \pm 0.001$	$0.130^{c,x} \pm 0.002$	$0.142^{d,x} \pm 0.001$	$0.193^{e,x} \pm 0.008$
7th day						
α-aminoacidic nitrogen, mg Leu/g	$130.16^{b,z} \pm 0.59$	$136.70^{c,z} \pm 0.64$	$137.66^{c,y} \pm 0.68$	$143.81^{d,z} \pm 0.68$	$122.08^{a,y} \pm 0.39$	$157.27^{e,z} \pm 0.76$
Protein carbonyls, nmol DNPH/mg protein	$0.230^{d,y} \pm 0.006$	$0.146^{a,y} \pm 0.001$	$0.188^{b,y} \pm 0.015$	$0.205^{c,y} \pm 0.008$	$0.241^{d,y} \pm 0.011$	$0.271^{e,y} \pm 0.009$
14th day						
α-aminoacidic nitrogen, mg Leu/g	$110.61^{a,x} \pm 0.44$	$114.26^{a,x} \pm 0.30$	$109.64^{a,x} \pm 0.43$	$103.97^{a,x} \pm 0.28$	$101.82^{a,x} \pm 0.47$	$106.12^{a,x} \pm 0.42$
Protein carbonyls, nmol DNPH/mg protein	$0.268^{c,z} \pm 0.006$	$0.216^{a,z} \pm 0.003$	$0.219^{a,z} \pm 0.003$	$0.245^{b,z} \pm 0.008$	$0.265^{c,z} \pm 0.014$	$0.269^{c,y} \pm 0.020$

cooked sausages with half-reduced nitrites to some extent (Table 3). Furthermore, nitrite reduction led to increased protein oxidation. The antioxidant effect of nitrites acts by a similar mechanism to the formation of the pink-red color of the cooked meat products (Lavado et al., 2021) where the formed nitriso- and nitrosyl compounds, particularly NOMb, have antioxidant properties (Hernández et al., 2021). This effect was observed in AN75 and previously reported by Vlahova-Vangelova et al. (2014).

CONCLUSION

The incorporation of three-component antioxidant blend slowed down the hydrolysis of the triglycerides and phospholipids in free fatty acids. The lipid fraction was stabilized by the inhibition of the lipid peroxidation and the accumulation of its end products. The cooked sausages prepared with three-component antioxidant blend underwent minor changes in protein fraction and have higher antioxidant potential.

The obtained results allowed us to conclude that the incorporation of the three-component antioxidant blend containing X_1 – 0.100 g/kg freeze-dried extract of dried distilled rose (*Rosa damascena* Mill.) petals, X_2 – 0.091 g/kg dihydroquercetin isolate from *Larix sibirica* Ledeb and X_3 – 0.100 g/kg sodium L-ascorbate can be used successfully for the production of functional cooked sausages with half-reduced nitrite content.

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