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ORIGINAL RESEARCH PAPER

COLOR STABILITY OF COOKED PORK-BEEF WIENER WITH REDUCED NITRITE AND NATURAL ANTIOXIDANTS FROM ROSE (*ROSA DAMASCENA* MILL.) PETAL EXTRACT

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Abstract

The polyphenol-rich extract from the spent rose (*Rosa damascena* Mill.) petals (SRPE) was used as natural antioxidant to sustain color stability in cooked beef-pork wiener with reduced nitrites. The cooked pork-beef wiener samples were evaluated during 7-day refrigerated storage at 0 + 4°C, as follows – positive control - 100 mg $NaNO_2$/kg; negative control - 50 mg $NaNO_2$/kg; RE33 33 mg extract/kg + 50 mg $NaNO_2$/kg; and RE66 - 66 mg extract/kg and 50 mg $NaNO_2$/kg. Significant recipe-storage time interaction was assessed in pH, instrumental color, % inhibition of DPPH, FRAP assay, peroxide value and 2-thiobarbituric acid reactive substances (TBARS). The incorporation of extract doesn't compromise the sensory profile such as taste, smell, and texture. The three samples with half-reduced nitrite had lower redness (a*) compared to the positive control. The weakest discoloration was observed in R66 during 7-day refrigerated storage (0+4°C). Compared to control RE66 was the most stable concerning peroxide value and TBARS. Even the reported strong antioxidant activity of SRPE, the lower concertation – RE33, wasn't able to inhibit the oxidation and discoloration compensating the nitrite reduction, as much as the higher dose – RE66. The antimicrobial activity of the SRPE was not so prominent yet at the end of cold storage R33 and R66 exhibited lower total plate count compared to both controls. Even the reported strong antioxidant activity of FDRPE, the results confirm that the lower concertation, wasn't able to inhibit the oxidative processes and discoloration and compensated the half reduction of nitrite as much as the higher dose.

Keywords*:* natural antioxidants; food safety; oxidation; polyphenols; meat processing

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Introduction

Nitrites (E249, E250) are among the most commonly used additives in the production of meat products, including emulsion-type sausages. Their effectiveness in ensuring microbiological safety, color stability and preventing lipid oxidative spoilage has been well documented (Lavado *et al.*, 2021; Moraru Manea *et al.*, 2025). They inhibit the development of dangerous pathogens such as *Clostridium botulinum*, the causative agent of botulism (Hui, 2012; Kim *et al.*, 2025).

In addition, nitrites contribute to the formation of the characteristic reddish-pink color of thermally processed meat products by interacting with myoglobin (Kureljušić *et al.*, 2025). Despite these functional benefits, the scientific community and health institutions have expressed serious concerns regarding the risk associated with excessive intake of nitrites, particularly due to the possibility of the formation of carcinogenic N-nitroso compounds (NOCs) in food and in the gastrointestinal tract (Andrade *et al.*, 2024; Lavado *et al.*, 2021). Among the most studied are N-nitrosodimethylamine (NDMA), N-nitrosopiperidine (NPIP) and N-nitrosopyrrolidine (NPYR), which have been associated with an increased risk of various types of cancer, including stomach, liver and esophageal cancer (Jo *et al.*, 2020; Huang *et al.*, 2025).

In this context, there is growing interest in alternative strategies that allow the reduction or complete replacement of nitrites while maintaining the quality and safety of meat products (Kolev and Baleva 2022; Huang *et al.*, 2025). In recent years, numerous studies have investigated biologically active compounds of natural origin, including plant extracts, essential oils, polyphenols, antimicrobial peptides and organic acids that have antioxidant and/or antimicrobial activity (Andrade *et al.*, 2024; Li *et al.*, 2025). Such approaches align with consumers' demand for clean-label products, minimal use of synthetic additives and a healthier food profile (Kim *et al.*, 2025; Moraru Manea *et al.*, 2025).

The spent rose (*Rosa damascena* Mill.) petals' extract, contains wide spectrum of polyphenolic compounds making it a suitable to serve as natural antioxidant in food processing (Dinkova *et al.*, 2022).

The present study explores the possibilities of reducing the nitrite content in cooked pork-beef wiener through the addition of biologically active components and evaluates their color stability, sensory profile, oxidative changes, antioxidant properties and effect on the microbial status during 7-day refrigerated storage (0 + 4°C). In the context of healthier nutrition and innovation in the food industry, such approaches represent a promising strategy for developing safer and natural meat products that meet modern market requirements.

Materials and methods

Materials

For the purpose of the experiment a pork-beef cooked wiener was produced using - pork bacon 60%, lean beef meat 40%, table salt - 2%; sodium nitrite ($NaNO_2$, E250), spices: black pepper (*Piper nigrum*) 0.400% and nutmeg (*Myristica fragrans*) - 0.100%, white crystal sugar - 0.100%.

The pork bacon and lean beef meat were purchased from the local retailer.

Table salt, $NaNO_2$, spices and sugar were purchased from (PI – 1 Ltd., Botevgrad, Bulgaria). Wiener was stuffed in polyamide casings supplied by "Excellpack Bulgaria" Ltd. The cooked and cooled wiener were packed in group packaging - bags made of multilayer moisture - and gas-tight transparent foil, which were sealed hermetically and stored at 0 +4℃ for 1 and 7 days.

The freeze-dried extract of *Rosa damascena* Mill. spent petals (SRPE) was prepared according to the procedure described by Vlahova-Vangelova *et al.* (2025).

Plan of the experiment

Samples were formulated as follows: CP – positive control with the addition of 100 mg/kg $NaNO_2$ without SRPE; CN – negative control with the addition of 50 mg/kg $NaNO_2$ without SRPE; RE33 – experimental sample with the addition of 33 mg freeze-dried extract of spent rose (*Rosa damascena* Mill.) petals (SRPE)/kg and 50 mg/kg $NaNO_2$; RE66 –– experimental sample with the addition of 66 mg freeze-dried extract of spent rose (*Rosa damascena* Mill.) petals (SRPE)/kg and 50 mg/kg $NaNO_2$; The cooked beef-pork wiener were produced as presented in Figure 1.

Methods

The preparation of the samples for the analyses of the physicochemical and microbiological parameters was carried out by homogenizing the wiener from average laboratory samples (Esbensen and Wagner 2014). The average laboratory samples were tested immediately after their preparation. The determination of the sensory and color properties of the samples did not require the preparation of average laboratory samples

Proximate composition

Moisture content was determined by drying at 104 - 105℃ until constant weight was reached (ISO 1442: 2023). Protein content was determined by determining the amount of total nitrogen determined by the Kjeldahl method (AOAC 1996). The amount of total protein was calculated using a nitrogen-protein conversion factor = 6.25. Total fat was determined using a Soxtherm apparatus (Gerhardt Analytical Systems, Germany) (ISO 1444:1996). Total ash content was determined by incinerating at 400 - 600℃ (ISO 936:1998). The amount of carbohydrates was calculated by subtracting from 100, the sum of the moisture, protein, fat and total ash content.

Sensory profile

A five-point hedonic score scale was used in the sensory analysis (Civille *et al.*, 2016). The following sensory characteristics were determined: appearance, color of the cut surface, texture, smell, taste. For each of the sensory characteristic, the each of the seven panelists could award from 1 to 5 points. The sensory perceptions of the panel were checked using a triangular test according to Sinkinson (2017). The testing was carried out on the basis of Art. 28(4) of the Regulations for the Organization of

Educational Activities at University of food technologies - Plovdiv and dean order No. 130 of 20.02.2025.

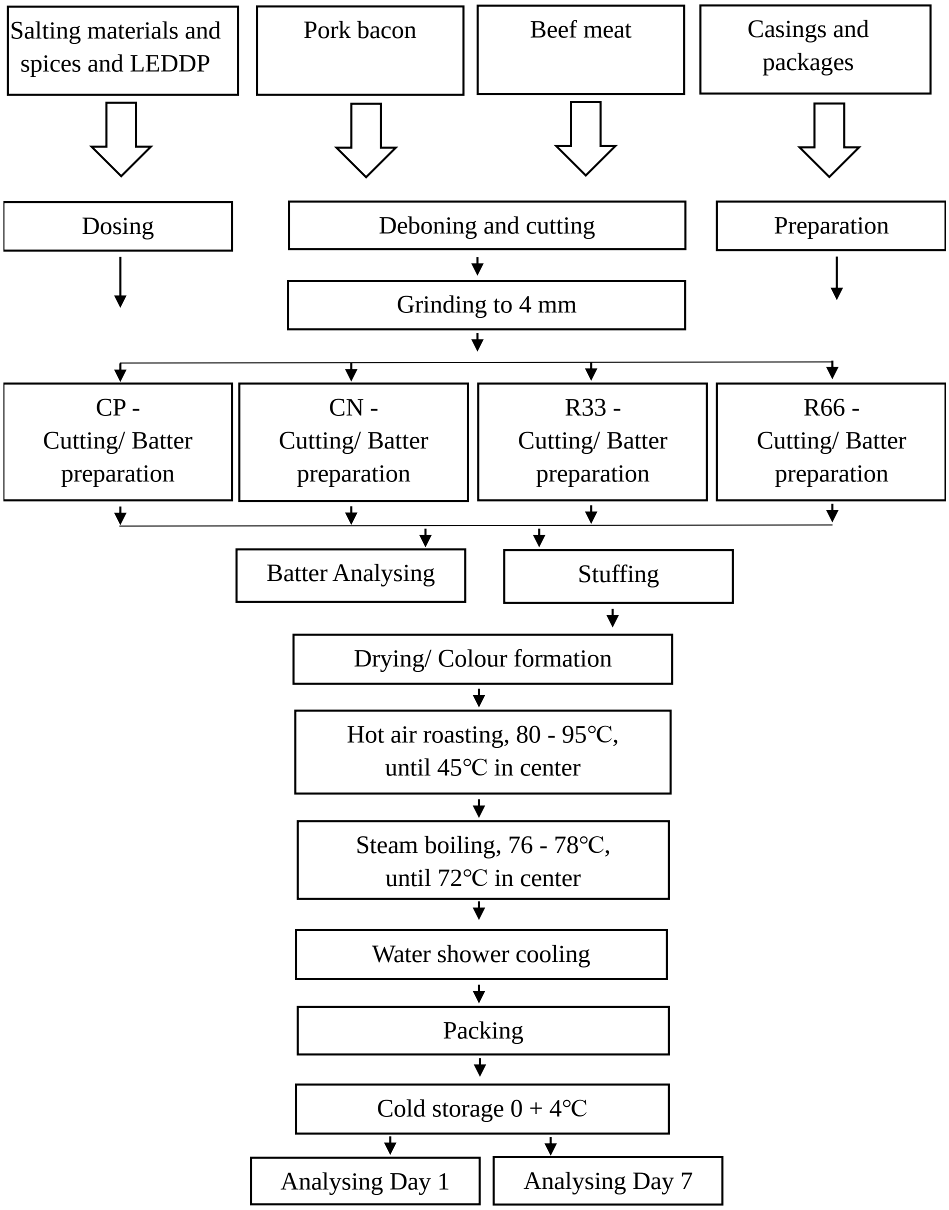

Figure 1. Flow diagram for the production of cooked wiener.

pH value

The pH value analyses were performed with a portable Hanna pH meter, model HI99163 (Hanna Instruments, Smithfield, USA), equipped with a meat contact sensor tip type FCO99. The device was calibrated with certified buffer solutions with pH 4.04 and 6.86 before each use (Kolev and Baleva, 2022).

Instrumental color

A Konica Minolta colorimeter CR-410 (Konica Minolta Holding, New Jersey, USA) was used to assess the lightness (L*), redness (a*) and yellowness (b*) on the cooked wiener' cross-cut surface (King *et al*., 2023).

Hydrolytic and oxidative changes in lipid fraction

The amount of free fatty acids (FFA) was determined according to ISO 660:2020 described by Prasad and Sivamani (2021). The peroxide value (PV) was determined spectrophotometrically (Shantha and Deeker 1994). The 2-thiobarbituric acid reactive substances (TBARS) were determined by the method of Botsoglou *et al.* (1994) modified by Moraru Manea *et al*. (2025).

Total phenolic content and antioxidant activity

Extraction of bioactive compounds was performed according procedure of Maria do Socorro *et al.* (2010). The total phenolic content (TPC) was determination is based on the reaction of Folin–Ciocalteu reagent and phenolic compounds described by Vardakas *et al*. (2024). The radical scavenging activity against 1,1-diphenyl-2-picrylhydrazil (DPPH) radical is performed according to Brand-Williams *et al.* (1995), with modifications by Dinkova *et al.* (2014). While the transition metals-chelating activity against ferric (Fe^{3+}) ions - FRAP assay of Benzie and Strain (1996) modified by Dinkova *et al.* (2014).

Microbial status

Sample preparation of the main suspension for microbiological studies was carried out according to ISO 6887-2:2017. For the determination of total aerobic mesophilic microorganisms – total viable count (TVC), the method of depth cultivation in Plate Count Agar (Merck, Germany) and incubation at 30℃ for 72 h was used, described in ISO:4833:2011/A1:2022. For the determination of colony-forming units of the *Enterobacteriaceae*, depth cultivation in Violet Red Bile Dextrose Agar (Merck, Germany) and incubation at 37℃ for 72 h was performed, according to ISO 21528-2:2017. The molds and yeasts (M&Y) quantification were determined by surface plating on Sabouraud dextrose agar and incubation at 20℃ for 72 h (ISO 21527-1:2008).

Data analysis

The statistical analysis of the obtained data was performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC). The PROC GLM procedure (Generalized Linear Model) with post-hoc comparison according to Tukey was used. Comparisons of the statistical significance of the differences in the mean values for each studied indicator affected by the modification of the recipe (M) and the storage time (S) of the cooked pork-beef wiener were made. The established differences were considered significant at $P < 0.05$ (Boykin *et al*., 2010).

Results and discussion

Technological characteristics of raw batter

No significant influence on the batter lightness (L*) was evaluated (Table 1). The modification of the formulation influenced ($P \leq 0.0001$) only the redness (*a**) of the raw batter color (Table 1). The incorporation of 66 mg SRPE/kg or 33 mg SRPE/kg and ½ reduction of $NaNO_2$ content led to increase of *a**-values compared to both positive (CP) and negative (CN) control. In the same time batter yellowness (*b**) was not influenced by the modification of the recipe.

Table 1. Instrumental color and pH of raw batter.

	*L**	*a**	*b**	pH
CP	67.13^{a}	6.63^{c}	12.48^{a}	6.20^{a}
CN	66.14^{a}	6.92^{b}	12.34^{a}	6.20^{a}
RE33	66.56^{a}	7.24^{a}	12.62^{a}	6.20^{a}
RE66	66.91^{a}	7.09^{ab}	12.49^{a}	6.20^{a}
RMSE*	0.442267	0.080829	0.178279	0.050000
P - value	*0.1029*	*< 0.0001*	*0.3405*	*1.0000*

The results are presented as mean values; a.b,c index statistically significant ($P \leq 0.05$) differences for the studied indicator; *RMSE – Root Mean Squared Error

The decrease of redness(browning) after addition of $NaNO_2$ to raw meat batter is well-known phenomena. King et al. (2023) described the reaction of rapid browning occurring when NO_2^{-} is added to fresh meats batters. Subsequently, under anaerobic conditions inside the casings, brown NO-MMb is reduced to red NO-myoglobin by added reductants such as the disaccharides, or by endogenous reductants, such as MMb reductase enzymes. During subsequent thermal processing, denturation of NO-myoglobin during cooking or exposes the centrally located porphyrin ring, resulting in the pink cured meat pigment nitrosyl-hemochrome, due to the interaction between ferrous iron and NO (Kureljušić *et al.*, 2025).

Proximate composition of cooked wiener

The proximate composition of the cooked wiener consisted of 65% moisture content, 18% fat, 15% protein (Figure 2). The addition of SRPE at levels of 33 or - 66 mg/kg was minimal and did not significantly affect the proximate composition of the products.

For comparison, Collins *et al.* (2018) reported 52.61% moisture, 22.95% fat and 10.64% protein in pork smoked sausages prepared with 0, 4 or 7-day pre-blends and stored in a refrigerator for up to 160 days under fluorescent lighting. Similarly, the proximate composition of different salami formulations was: moisture (60.46 – 63.28%), proteins (11.31 – 12.65%), fats (19.11 – 22.02%) (Moraru Manea *et al.*, 2025). The present results showed higher protein and lower fat content compared to both of the above-mentioned studies possibly due to the fact that they are evaluating pork formulations, not a pork-beef as in our case. According to Hui (2012), cooked and smoked sausages account for approximately 85% of all sausage production today. All cooked and smoked meat products in the sausage, frankfurter and wiener categories should contain up to 30% fat (Hui, 2012).

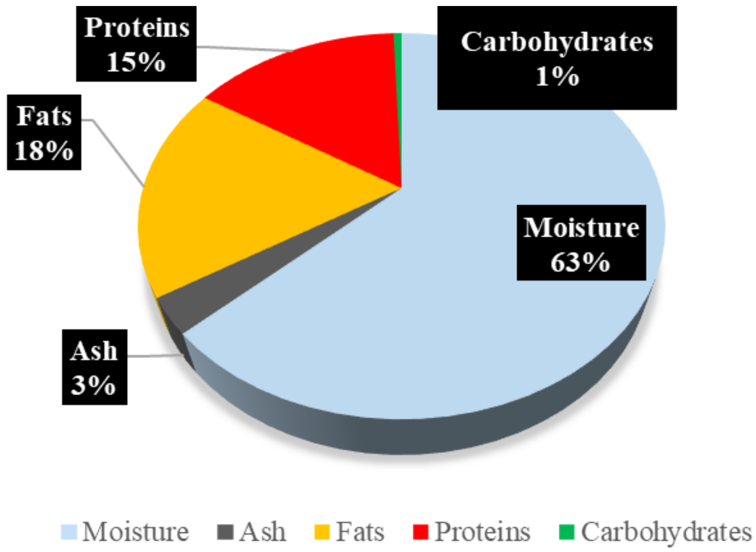

Figure 2. Proximate composition of cooked wiener.

Sensory profile

The positive control CP cooked wiener was evaluated with the highest sensory scores for all sensory attributes (Figure 3), and those of the negative control CN with the exception of the taste attribute. Sensory scores for all sensory-characteristics (Figure 3) of the experimental samples RE66 and RE33 occupy intermediate values, with a slightly pronounced advantage for samples RE66. The described differences are especially clearly expressed for the indicator color of the cut surface with more than 1 point. The results demonstrated that the addition of 66 mg/kg or 33 mg/kg of freeze-dried extract of spent rose (*Rosa damascena* Mill.) petals accompanied by ½ reduced $NaNO_2$ content (addition of 50 mg/kg $NaNO_2$) (samples RE66 and RE33) significantly improves the sensory characteristics of the cooked wiener compared to the negative control CN. However, the sensory quality achieved with 100 mg/kg $NaNO_2$ (CP) was not fully attained. The results thus obtained allow us to conclude that the addition of 66 or 33 mg SRPE/kg to the batter for winers with ½ reduced $NaNO_2$ content does not essentially affect the appearance, consistency, smell and taste of the sausages, but leads to a less favorable perception of the color of the cut surface. This can be explained by the multifunctional properties of the $NaNO_2$, due to which their replacement in cured meat processing is a challenge. Nitrites function as color-forming agents, flavor contributors, antioxidants, and antimicrobial compounds (Kureljušić *et al*., 2025).

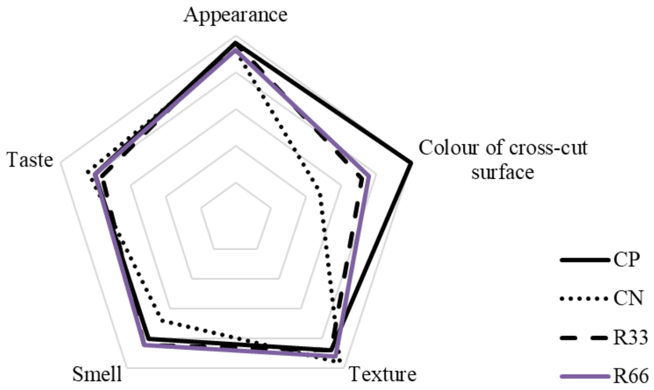

Figure 3. Sensory profile of cooked wiener.

Our results confirm the findings of Lee *et al.* (2017), who reported no significant differences in texture between sausage samples with or without the addition of $NaNO_2$. Similar, Kolev and Baleva (2022 hypothesized that the mixture containing 0.100 g/kg freeze-dried rose petal extract (SRPE); 0.091 g/kg dihydroquercetin and 0.100 g/kg sodium L-ascorbate successfully replaces sodium nitrite up to 50% without significantly changing the sensory attributes of the sausages.

Instrumental color

It was found that the interaction of the factors: addition of 66 mg SRPE /kg or 33 mg SRPE /kg and 50 mg/kg $NaNO_2$, as well as refrigerated storage time significantly affected ($P \leq 0.05$) all three color components: brightness (L^*), red color component (a^*) and yellow color component (b^*) of the stored sausages (Table 2). The redness (a^*) of the cooked wiener differed significantly, primarily between the positive control (CP) and the remaining three samples CN, RE33 and RE66 (Table 3). The addition of 66 mg SRPE /kg or 33 mg SRPE /kg and 50 mg/kg $NaNO_2$ resulted in a reduction of the red component (a^*) on both day 1 and 7 of refrigerated storage (Table 3). This reduction was more pronounced at the lower dose (33 mg SRPE /kg) and ranging from 27.7% to 22.7% (Table 2). The reduction of a^* color component in sample RE66 was 23.4 and 16.3%, respectively, compared to CP. Plant extracts from the *Rosaceae* family are known to be rich in polyphenolic compounds, which are considered functional ingredients with the potential to inhibit pigment oxidation (Dinkova *et al.*, 2022). However, the presented results indicate that the addition of 33 mg SRPE/kg and to a lesser extent 66 mg SRPE/kg to the filling mass of sausages with half-reduced $NaNO_2$ content causes a reduction in a^* values of the cooked wiener without significantly affecting the b^* and L^* during storage (Table 2). Regarding the yellow color component (b^*), the largest statistically significant difference was between the negative control sample with the addition of 50 mg/kg $NaNO_2$ without SRPE (CN) and the remaining three samples CP, RE33 and RE66 (Table 3). Throughout the experimental period, it was noted that the interaction of the two factors was significant ($P \leq 0.0121$). On day 7 of refrigerated storage, the b^* values were significantly higher than on day 1 for all samples. This suggests that the lower concentration (< 50 mg/kg) of added $NaNO_2$ is not sufficient to bind the available myoglobin, as well as the formation of NO-Mb. Several authors report that the remaining "free" myoglobin undergoes oxidation to metmyoglobin – browning of the color, which is a likely cause of the increased *b** values (Suman and Joseph, 2013; Armenteros *et al.*, 2016; King *et al.*, 2023). Similar higher *b** values were also reported in cooked sausages with 100% nitrite reduction and addition of rosemary and green tea extract (Yoon *et al.*, 2021).

Table 2. Instrumental color cooked wiener during 7-day refrigerated storage (0 + 4℃).

	*L**		*a**		*b**	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
CP	65.08^{ab}	63.91^{b}	9.85^{a}	8.43^{b}	8.63^{de}	9.65^{bc}
CN	64.70^{ab}	64.60^{ab}	7.72^{c}	6.34^{e}	9.03^{cd}	10.97^{a}
RE33	64.94^{ab}	65.45^{a}	7.12^{cd}	6.54^{e}	8.68^{de}	9.73^{b}
RE66	64.18^{ab}	64.49^{ab}	7.50^{cd}	7.06^{d}	8.16^{e}	9.68^{bc}
RMSE*	0.330252		0.236300		0.238118	

	M	S	M×S	M	S	M×S	M	S	M×S
P - value	0.0021	0.4096	0.0020	< 0.0001	< 0.0001	0.0029	< 0.0001	< 0.0001	0.0121

The results are presented as mean values; a, b, c, d, e index statistically significant ($P \leq 0.05$) differences for the studied indicator; *RMSE – Root Mean Squared Error

The instrumental color (*L**, *a**, *b**) are consistent with the established less favorable perception of the color of the cut surface from the sensory analysis of the cooked wiener, with the exception of the taste indicator. A similar decrease in color brightness *L** has been reported in pork burgers with added wild grape extract (*Ampelopsis grossedentata*) (Zhang *et al.*, 2019), as well as in pork frankfurters with incorporated rosehip extract (*Rosa canina* L.) (Vossen *et al.*, 2012). Identical trend in redness of Chinese sausages was reported by Huang *et al.* (2025). They evaluated a lower redness at day 0 in sausages with ½ nitrite reduction compared to control, as well as a decrease during 7-day storage. In their study, the control sample (N120) showed a greater decline in a* values than the TR200+N60 treatment, suggesting that polyphenol extracts may help stabilize color in sausages with partial nitrite reduction. Kolev and Baleva (2022) similarly reported that sausages produced with a mixture of a ternary mixture (0.100 g/kg freeze-dried extract of spent rose petals (*Rosa damascena* Mill.); 0.091 g/kg dihydroquercetin isolate from *Larix sibirica* Ledeb and 0.100 g/kg sodium L-ascorbate) were characterized by lower color brightness (*L**) compared to the control. Although the intensity of the red color component (*a**) in sausages produced with the addition of 100, 75 and to some extent 50 mg/kg $NaNO_2$ was higher than that measured in the control, the oxidation of nitrosohemochrome to methemochrome and the increase in the yellow color component (*b**) was greatest in sausages without the addition of nitrites. Grispoldi *et al*. (2022) demonstrated that the use of natural antioxidants, such as sorghum husk extract (*Sorghum bicolor*), largely preserved the original characteristics of canned meat products. Stabilization of oxidative processes mainly instrumental color of pork frankfurters was also achieved by addition of catechin. Li *et al.* (2025) report a significantly improved the *L**-, *a**-, and *b**-values of frankfurters. They suggest that the evaluated discoloration is likely due to the oxidation of pigment proteins, due to formation of free radicals generated by the lipid peroxidation. To stabilize the instrumental color (*L**, *a** and *b**) of Chinese sausages with ½ $NaNO_2$ content, Huang *et al.* (2025) suggest using 200 mg/kg of thearubigins. They are which are polyphenolic compounds found in black tea, formed during the enzymatic oxidation of catechins such as epigallocatechin gallate (EGCG) and epicatechin (EC).

pH value

It was found that the interaction: the addition of 66 mg SRPE /kg or 33 mg SRPE /kg and ½ reduction of the $NaNO_2$ content influence ($P \leq 0.0002$) the pH value of the sausages stored for 7 days at 0 - 4°C (Table 3). The statistical analysis showed that the refrigerated storage time alone did not have a statistically significant effect on the pH value of the sausages ($P \geq 0.4774$).

Table 3. pH value of cooked wiener during 7-day refrigerated storage (0 + 4°C).

	pH		
	Day 1		Day 7
CP	6.07^{c}		6.20^{b}
CN	6.20^{b}		6.23^{b}
RE33	6.27^{ab}		6.20^{b}
RE66	6.37^{a}		6.22^{b}
RMSE*	0.042081		
P - value	M	S	M×S
	< 0.0001	*0.4774*	*0.0002*

The results are presented as mean values; a, b, c index statistically significant ($P \leq 0.05$) differences for the studied indicator; *RMSE – Root Mean Squared Error

After one day of refrigerated storage, the addition of 66 mg SRPE /kg and 33 mg SRPE /kg to ½ reduction of $NaNO_2$ resulted in a slight increase in pH compared to the positive control sample CP, ranging from 3.2 to 4.7% (Table 3). Overall, the results indicate that incorporation of 66 or 33 mg SRPE/kg to the batter of sausages with ½ reduced $NaNO_2$ content essentially does not affect the pH value. These findings confirm the reported no significant change of pH during 14-day storage at 4 °C of frankfurters prepared with lard and catechin (Li *et al.*, 2025). At the same time, Yoon *et al.* (2021) reported a significant decrease in pH values after a 100% reduction in added sodium nitrite, accompanied by the addition of green tea extract and rosemary in pork sausages.

Hydrolytic and oxidative changes in lipid fraction

The incorporation of 66 mg SRPE /kg or 33 mg SRPE /kg into wiener with half-reduced $NaNO_2$ content did not influence ($P > 0.05$) the levels of free fatty acids, expressed as % oleic acid. During 7-day refrigerated storage (0+4°C), an increase in FFA was found in all samples (Table 4). This indicates that the addition of SRPE and the reduction of the nitrite content of the sausages do not have a direct effect on the course of lipolytic changes in the lipid fraction of this type of cooked-smoked meat products. In contrast, Kolev *et al.* (2022) reported that inclusion of three-component antioxidant mixture in the composition of cooked sausages, slowed the hydrolysis of triglycerides and phospholipids into free fatty acids. The lipid fraction is stabilized by inhibiting lipid peroxidation and accumulation of its secondary end products. Similarly, the acid value in Chinese sausages with thearubigins and nitrite reduction increase during 14-day of storage (Huang *et al.*, 2025).

Table 4. Hydrolytic and oxidative changes of cooked wiener during 7-day refrigerated storage (0 + 4°C).

<table>
<tr><td></td><td colspan="3">FFA, % oleic acids</td><td colspan="3">POV, meq 0$_2$/kg</td><td colspan="3">TBARS, mg MDA/kg</td></tr>
<tr><td></td><td colspan="2">Day 1</td><td>Day 7</td><td colspan="2">Day 1</td><td>Day 7</td><td colspan="2">Day 1</td><td>Day 7</td></tr>
<tr><td>CP</td><td colspan="2">0.88^{b}</td><td>1.00^{a}</td><td colspan="2">1.75^{a}</td><td>1.24^{ab}</td><td colspan="2">1.66^{b}</td><td>2.44^{a}</td></tr>
<tr><td>CN</td><td colspan="2">0.88^{b}</td><td>0.94^{a}</td><td colspan="2">1.84^{a}</td><td>0.52^{b}</td><td colspan="2">1.75^{b}</td><td>2.64^{a}</td></tr>
<tr><td>RE33</td><td colspan="2">0.81^{b}</td><td>1.07^{a}</td><td colspan="2">1.82^{a}</td><td>1.34^{ab}</td><td colspan="2">1.57^{b}</td><td>2.46^{a}</td></tr>
<tr><td>RE66</td><td colspan="2">0.74^{b}</td><td>1.08^{a}</td><td colspan="2">1.60^{ab}</td><td>1.27^{ab}</td><td colspan="2">1.04^{d}</td><td>1.31^{c}</td></tr>
<tr><td>RMSE*</td><td colspan="3">0.212750</td><td colspan="3">0.128436</td><td colspan="3">0.079739</td></tr>
<tr><td rowspan="2">*P* - value</td><td>M</td><td>S</td><td>M×S</td><td>M</td><td>S</td><td>M×S</td><td>M</td><td>S</td><td>M×S</td></tr>
<tr><td>0.9850</td><td>0.0378</td><td>0.6869</td><td>0.0004</td><td>< 0.0001</td><td>< 0.0001</td><td>< 0.0001</td><td>< 0.0001</td><td>< 0.0001</td></tr>
</table>

The results are presented as mean values; a, b, c, d index statistically significant ($P \leq 0.05$) differences for the studied indicator; *RMSE – Root Mean Squared Error

The interaction, between formulation (addition of SRPE with a 50% reduction in $NaNO_2$ content) and storage time significantly affected the peroxide value (PV) of all sausage samples ($P \leq 0.0001$) (Table 4). The lowest PV ($P \leq 0.05$) values, recorded in the negative control sample CN on the seventh day of refrigerated storage are likely attributable to faster degradation of hydroperoxides relative to their formation (Domínguez *et al.*, 2019). In the absence of SRPE, the processes of degradation of hydroperoxides to low molecular weight secondary products are more accelerated. Overall, the concentration of lipid hydroperoxides, expressed as PV, remained relatively low in all samples throughout the 7-day storage period and did not exceed 2.00 meq O_2/kg (Table 4). This suggests that the formation and transformation of primary products of lipid peroxidation is in a certain chemical equilibrium. In contrast, a different trend was observed for secondary lipid oxidation products measured as TBARS. Compared to the positive control sample CP, the addition of 66 mg SRPE/kg at ½ reduced $NaNO_2$ content to the cooked wiener statistically significantly ($P \leq 0.05$) slowed down the increase in the TBARS of lipids, both after one day and after seven days of refrigerated storage at 0 + 4°C (Table 4). It should be noted that TBARS in all samples remained well below the threshold of 3.00 mg MDA/kg, which is commonly considered the upper limit associated with significant oxidative deterioration and potential quality or safety concerns (Domínguez *et al.*, 2019; Li *et al.*, 2025). For a similar effect, Kolev *et al.* (2022) suggest including a three-component antioxidant mixture containing 0.100

g/kg freeze-dried extract of spent rose petals (*Rosa damascena* Mill.); 0.091 g/kg dihydroquercetin isolate from *Larix sibirica* Ledeb and 0.100 g/kg sodium L-ascorbate. Similarly, Huang *et al.* (2025) demonstrated stabilization of oxidative processes in Chinese sausages by **s**ubstituting sodium nitrite with thearubigins They reported a significant inhibition of TBARS formation after incorporation of 200 and 300 mg/kg of thearubigins and ½ and ¼ nitrite reduction, respectively. Another both dose-dependent and species-dependent inhibition of oxidative processes and improvement of lipid stability was reported in different nitrite-free salami formulations using fruit powders: sour cherry, blackcurrant and lingonberry (Moraru Manea *et al.*, 2025).

Total phenolic content and antioxidant activity

The addition of 66 mg SRPE/kg at a half-reduced $NaNO_2$ content in the cooked wiener increased their total phenolic content statistically significantly ($P \leq 0.05$) compared to both the positive control sample CP and the negative control sample CN (Table 5). After one day of refrigerated storage (0+4°C) of the cooked wiener, sample RE66 contained more total phenolics compared to both of the positive and negative control sample CP and CN, respectively 59.2% and 62.3% (Table 5). The 7-day refrigerated storage (0+4°C) did not influence ($P \geq 0.3065$) the total phenolic content in all examined samples (Table 5).

Table 5. Total phenolic content (TPC) and antioxidant activity of cooked wiener during 7-day refrigerated storage (0 + 4°C).

	TPC, mg GA/kg			% Inhibition of DPPH			FRAP, µmol TE/kg		
	Day 1		*Day 7*	*Day 1*		*Day 7*	*Day 1*		*Day 7*
CP	980.8^{bx}		1040.0^{bx}	67.4^{d}		84.6^{a}	601.2^{a}		304.1^{b}
CN	961.9^{bx}		1002.4^{bx}	65.8^{d}		83.7^{ab}	598.3^{a}		289.9^{b}
RE33	937.9^{bx}		737.4^{cx}	72.3^{cd}		73.8^{cd}	524.0^{a}		222.5^{b}
RE66	1561.1^{ax}		1407.7^{ax}	78.9^{abc}		79.2^{abc}	671.4^{a}		565.5^{a}
RMSE*	135.790010			3.670104			60.581022		
P value	M	S	M×S	M	S	M×S	M	S	M×S
	< 0.0001	0.3065	0.2355	0.0693	< 0.0001	0.0005	< 0.0001	< 0.0001	0.0276

The results are presented as mean values; a, b, c, d, ax, bx, cx index statistically significant ($P \leq 0.05$) differences for the studied indicator; *RMSE – Root Mean Squared Error

The antioxidant activity of the wieners against the DPPH radical was influenced by the interaction of the two studied factors, as well as by the factor of refrigerated storage time (Table 5). Compared to the two control samples CP and CN, the addition of 66 mg SRPE/kg and 33 mg SRPE/kg in half reduced $NaNO_2$ content wiener increased the % inhibition of the DPPH radical after one day of refrigerated storage (0+4°C). However, after seven days of storage, DPPH inhibition values in the SRPE-treated samples were lower by 5.4% (RE66) and 10.8% (RE33), respectively, compared with day 1 values (Table 5). The significant increase in DPPH inhibition observed in the control samples (CP and CN) after seven days of refrigerated storage may be attributed to ongoing hydrolytic and oxidative processes. Accumulation of highly reactive lipid oxidation by-products or partial hydrolysis of the protein fraction resulting in the accumulation of peptides and amino acids may lead to an illusory higher % inhibition of the DPPH radical (Yu *et al.,* 2017; Domínguez *et al.,* 2019; Domínguez *et al.,* 2021;).

The antioxidant activity determined by the FRAP assay was influenced by the interaction ($P \leq 0.0276$). On day 1 of the refrigerated storage, all four samples did not demonstrate statistically significant differences (Table 5). Compared to the positive control sample CP, the addition of 66 mg SRPE/kg in half-reduced $NaNO_2$ wiener increased their FRAP antioxidant activity approx. 86%, compared to the negative control sample CN - by 95% (Table 5). The results obtained prove that with the addition of the higher dose of 66 mg/kg SRPE, the total phenolic content and the antioxidant activity determined by the FRAP assay in wiener with half the $NaNO_2$ content increased significantly, but the antioxidant activity of the sausages against the DPPH radical was not affected (Table 5). The absence of significant difference ($P \leq 0.05$) in the FRAP values among samples on day 1 is consistent previously reported antioxidant properties of $NaNO_2$, which are more pronounced against free radical scavenging than against the reduction of free iron ions (Jo *et al.,* 2020; Lavado *et al.,* 2021). Comparable findings were reported by Kolev *et al.* (2022) who observed a proportional reduction in residual nitrites in cooked sausages accompanied by increased DPPH antiradical activity and increased iron reduction potential (FRAP) with increasing doses of the added three-component antioxidant mixture. The observed effect can be explained by the specific chemical composition of spent rose petals, which contain three groups of polyphenolic compounds: kaempferol glycosides > quercetin glycosides > gallic acid glycosides. Approximately 30 polyphenolic antioxidant components have been identified in these extracts, contributing to their high antioxidant potential (Dinkova *et al*., 2022). The *Rosa damascena* petal extract (SRPE) used in the experiments exhibits strong antioxidant activity, effectively scavenging DPPH• and ABTS•+ radicals, with remarkable FRAP and CUPRAC values. The presence of phenolic compounds such as gallic acid and rutin, together with organic acids such as ascorbic acid, proves the hypothesis of its antioxidant and metal-chelating properties. These data prove SRPE as a potent natural antioxidant with potential applications in meat products (Kolev *et al.,* 2025).

Microbial status

The addition of 66 mg SRPE /kg or 33 mg SRPE /kg and ½ reduction of the $NaNO_2$ content did not affect the microbiological status of the cooked wiener in respect to *Enterobacteriaceae* family, molds and yeasts. These microorganisms were not detected in any of the tested samples during the 7-day refrigerated storage (0+4°C) of the sausages (Table 6). However, incorporation of 66 mg SRPE /kg into cooked wiener contributed to a statistically significant reduction in the total viable count (TVC) (Table 6) - by 0.35 lg KOE/g after one-day refrigerated storage and by 1.00 -

1.09 lg CFU/g - after 7-day storage. The results indicate that the addition of 66 mg SRPE/kg in cooked wiener produced with half $NaNO_2$ content exerts measurable antimicrobial activity, as reflected by the decrease in TVC (Table 6).

Table 6. Microbial status of cooked wiener during 7-day refrigerated storage (0 + 4°C).

	TVC, lg CFU/g			*Enterobacteriaceae*, lg CFU/g			M&Y, lg CFU/g		
	Day 1		Day 7	Day 1		Day 7	Day 1		Day 7
CP	3.50^{ay}		4.80^{ax}	Not found			Not found		
CN	3.50^{ay}		4.89^{ax}						
RE33	3.69^{ay}		3.80^{bx}						
RE66	3.15^{by}		3.80^{bx}						
RMSE*	0.325077			-			-		
P - value	M	S	M×S	M	S	M×S	M	S	M×S
	0.0402	0.0007	0.0734	-	-	-	-	-	-

The results are presented as mean values; ax, bx, ay, by index statistically significant ($P \leq 0.05$) differences for the studied indicator; *RMSE – Root Mean Squared Error

The absence of detectable Enterobacteriaceae, molds, and yeasts may be attributed primarily to adequate thermal processing and strict hygienic practices implemented under HACCP-based production systems, which effectively minimize post-processing contamination in this category of meat products. Therefore, potential treatment-related differences for these microbial groups could not be evaluated. The observed reduction in TVC may be associated with the antimicrobial activity of SRPE, thus helping to extend shelf life and control pathogenic contamination in sausages (Kolev *et al.*, 2025).

Conclusions

The results of the present study demonstrate that incorporation of 66 mg/kg freeze-dried extract from spent rose (*Rosa damascena* Mill.) petals (SRPE), combined with a 50% reduction in $NaNO_2$ content, represents a promising strategy for partial nitrite replacement in cooked pork–beef wieners. Although the reduced-nitrite formulations exhibited a less intense pink coloration of the cut surface compared with the full-nitrite control, the color stability during 7-day refrigerated storage (0–4 °C) was maintained, particularly at the higher SRPE dose. The increase in total phenolic content was associated with enhanced antioxidant capacity, as evidenced by improved FRAP values and significant inhibition of secondary lipid oxidation (TBARS), while peroxide values remained within low and acceptable limits. Importantly, all TBARS values were well below commonly accepted thresholds of oxidative deterioration. In contrast, incorporation of 33 mg/kg SRPE was insufficient to fully compensate for the technological functions of reduced nitrite, particularly regarding color intensity and oxidative stability.

Declaration of competing interest

The authors declare that there is no conflict of interest in writing this manuscript. All authors have contributed equally in the writing of this manuscript.

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