71st ICoMST
GIRONA - CATALONIA - SPAIN
3rd-8th August - 2025

S-NITROSOTHIOLS AS PROMISING NITRITE REPLACERS – COLOR FORMING AND STABILITY ASPECTS

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I. INTRODUCTION

The current trend of reducing nitrite content in meat products has led to the search for new and safe additives that can provide similar preservative, antioxidant, and color-enhancing functions. Nitrite plays a crucial role in preventing spoilage and ensuring the sensory quality of meat. A promising alternative group of compounds is nitrosothiols (RSNOs), which are naturally found in living organisms and serve as nitric oxide (NO) donors. These compounds form in vivo through reactions involving NO signaling pathways. They function as NO carriers and donors, providing a controlled release mechanism compared to unstable free NO. Some trials using RSNO in meat products have been already coducted (Andrade et al., 2024; Shpaizer, Kanner, & Tirosh, 2021). In the present study, three S-nitroso thiols were used as nitrosating agents: S-nitroso glutathione (G), S-nitroso acetylcysteine (A), and S-nitroso acetylcysteine ethyl ester (E). The aim of the study was to determine whether RSNOs added to meat are able to form acceptable and stable color throughout a certain storage period**.**

II. MATERIALS AND METHODS

Pork *m. quadriceps femoris* muscle was ground (plate Ø 3 mm, Mado, Germany), mixed, and divided into portions. Three S-nitroso thiols were used: S-nitroso glutathione (G), S-nitroso acetylcysteine (A), and S-nitroso acetylcysteine ethyl ester (E). RSNOs were applied as a freshly prepared aqueous solutions. All samples contained the same amount of water to ensure uniform RSNO distribution. Two control samples were prepared: one with water only (H_2O control) and one with sodium nitrite at 80 mg/kg (1.16 mmol/kg) (NO_2 control). Experimental samples contained RSNOs, at different molar equivalents relative to sodium nitrite: 1.16 mmol/kg (equivalent to NO_2), 0.60 mmol/kg (50% of NO_2 equivalent), 0.39 mmol/kg (33% of NO_2 equivalent), and 1.74 mmol/kg (150% of NO_2 equivalent). Each sample was homogenized (Robot Coupe, France) for three minutes, portioned (60 g), and placed in glass containers. The meat was cooked in a convection oven at 99°C for 30 minutes under 100% steam, then vacuum-packed and stored at 4°C for 21 days. The color analysis using CIELab (Konica Minolta CM-3500d, Osaka, Japan) was conducted on samples on the 1st and 21st day of storage. Statistical analysis was performed using two-way ANOVA analysis (Statistica 13, Tibco, Palo Alto, USA). Tukey test was performed to detect differences between means at $P< 0.05$.

III. . RESULTS AND DISCUSSION

The shelf life of meat products is determined by factors such as microbial stability, oxidative stability, and color stability. Food discoloration is the first quality trait visible to consumers and can lead to negative purchase decisions. Both oxidation and microbial activity affect color. Therefore, it is essential not only to achieve an acceptable color in meat samples but also to maintain this quality throughout the storage period (Tomasevic, Djekic, Font-i-Furnols, Terjung, & Lorenzo, 2021). The color parameters of the analyzed samples are presented in Table 1. As expected, the control sample containing water differed significantly in a* and b* coordinate values, showing the lowest and highest values, respectively. Significant differences in the a* parameter were observed between the water-containing sample and all other samples containing sodium nitrite or RSNOs at any concentration. The highest a* values on the first day after production were found in samples A1 and A1.5, which were higher than those in the NO_2 control sample. However, the redness stability of these samples was lower compared to NO_2. Sample E1.5 exhibited greater stability than NO_2, maintaining higher a* values on the 21st day of storage. Differences in lightness among the samples were minimal. G0.5 and E0.3 were the brightest samples, while G1.5 was the darkest on the first day of analysis. Storage time did not affect the b* coordinate in any of the tested samples. The yellowness of all tested samples (except for the H_2O sample) was comparable to that of NO_2.

Table 1. Color parameters in meat samples with RSNOs at various concentrations. Mean values ± standard errors.

Sample ID	Day of storage	L	a*	b*
H_2O	1	61.6^{abcde} ±0.5	3.8^{j} ±0.1	14.4^{a} ±0.1
	21	62.7^{a} ±0.4	4.0^{j} ±0.1	14.1^{a} ±0.1
NO_2	1	60.5^{cde} ±0.4	8.2^{abc} ±0.1	10.6^{de} ±0.2
	21	61.6^{abcde} ±0.4	7.3^{efgh} ±0.1	11.1^{cd} ±0.1
A1	1	59.0^{de} ±0.8	8.7^{a} ±0.1	11.4^{bcd} ±0.4
	21	59.9^{cde} ±0.5	6.3^{i} ±0.2	12.5^{b} ±0.2
A0.5	1	60.0^{cde} ±0.7	8.4^{abc} ±0.1	11.0^{cde} ±0.3
	21	59.9^{cde} ±0.5	6.7^{ghi} ±0.2	11.6^{bc} ±0.3
A0.3	1	59.8^{cde} ±0.7	8.1^{abcde} ±0.2	10.9^{cde} ±0.2
	21	60.1^{cde} ±0.3	7.1^{efghi} ±0.3	11.2^{cd} ±0.3
A1.5	1	59.9^{cde} ±0.4	8.7^{a} ±0.1	10.7^{cde} ±0.1
	21	59.6^{cde} ±0.4	6.5^{hi} ±0.2	11.4^{bcd} ±0.2
E1	1	62.3^{abcd} ±0.5	7.6^{bcdefg} ±0.2	9.9^{e} ±0.1
	21	61.5^{abcde} ±0.4	7.8^{bcdefg} ±0.2	10.5^{de} ±0.2
E0.5	1	61.7^{abcde} ±0.6	7.4^{cdefgh} ±0.3	10.4^{de} ±0.2
	21	60.7^{abcde} ±0.8	7.6^{bcdefg} ±0.1	10.5^{de} ±0.1
E0.3	1	63.0^{a} ±0.3	6.5^{hi} ±0.3	10.6^{de} ±0.3
	21	62.6^{abc} ±0.5	6.8^{defgh} ±0.1	10.7^{cde} ±0.3
E1.5	1	61.7^{abcde} ±0.4	8.6^{ab} ±0.2	10.1^{e} ±0.1
	21	61.0^{abcde} ±0.6	8.2^{abcd} ±0.1	10.5^{de} ±0.1
G1	1	62.3^{abcde} ±0.5	8.2^{abcd} ±0.1	10.0^{e} ±0.1
	21	61.3^{abcde} ±0.9	7.3^{defgh} ±0.1	10.5^{de} ±0.2
G0.5	1	63.2^{a} ±0.6	7.6^{bcdefg} ±0.1	9.9^{e} ±0.1
	21	62.2^{abcde} ±0.6	7.1^{efghi} ±0.2	10.8^{cde} ±0.2
G0.3	1	60.2^{cde} ±0.9	8.0^{abcde} ±0.1	10.1^{e} ±0.2
	21	61.4^{abcde} ±0.7	7.3^{defgh} ±0.2	10.7^{cde} ±0.1
G1.5	1	58.8^{e} ±0.9	8.0^{abcde} ±0.2	10.0^{e} ±0.1
	21	61.5^{abcde} ±0.5	7.1^{efghi} ±0.2	11.2^{cd} ±0.2

IV. CONCLUSION

All of the tested RSNOs at the molar concentrations equivalent or higher than NO_2 can be used as color-forming agents. Their a* coordinate values were comparable to those of the sample containing $NaNO_2$. However, the S-nitroso acetylcysteine ethyl ester provided the best color stability. Using RSNOs is promising as nitrite alternatives in terms of color forming agents.

ACKNOWLEDGEMENTS

This research was funded by the National Science Centre, Poland, Project No. 2022/45/B/NZ9/01840

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