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NITRIC OXIDE SYNTHASE ENZYME AS AN ALTERNATIVE CURING SYSTEM IN UNCOOKED, FERMENTED AND DRIED SALAMI

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I. INTRODUCTION

Consumer concerns regarding the safety of cured meat products made using nitrite have increased due to its potential to form carcinogenic N-nitrosamines. Previous research has suggested that the endothelial nitric oxide synthase enzyme activated by the amino acid L-arginine generates nitric oxide (NO) in post rigor skeletal muscle, suggesting a potential alternative curing system1 This study investigated the effects of pH on the endothelial nitric oxide synthase (eNOS) enzyme system's ability to generate nitric oxide and cured meat color in uncooked, fermented, and dried salami (UFD).

II. MATERIALS AND METHODS

Six 2.27kg batches of a pork/beef mix (60:40) (25% fat) were ground (12.7mm) and a salami preblend seasoning (A.C. Legg, Birmingham, AL, USA) with 1.25% sodium erythorbate, 2% salt, and 1% dextrose was added. Each batch was then treated with either 156 ppm sodium nitrite (Control) or L-arginine-HCl (Arg) at various concentrations of 1000 to 5000 pp. After hand mixing (2 min), a lactic acid starter culture (Saga 200, Kerry Group, Beloit, WI, USA) was diluted in 0.5% H_2O added to the batch, mixed, reground (4.76mm) and stuffed in 38mm fibrous casings (ViskoTeepak, Kenosha, WI, USA) to generate 30 chubs/treatment (0.22kg/chub). The chubs were fermented for 12 hrs at 37°C to a pH <5.0, then dried 3 days at 18°C and 80% RH and then for 8 days at 18°C and 70% RH until water activity <0.920 was achieved. Chubs were vacuum packaged with casings on and stored at 4.4°C until day of analysis (d 1, 14, 28 and 56 days of storage) for pH, residual nitrate (RNO3), residual nitrite (RNO2), nitrosylhemochromagen (NO-Heme), total pigment and water soluble pigment levels. The experiment was designed as a 5 (L-arginine concentrations) x 4 (storage periods) factorially arranged randomized complete block design with an augmented control, replicated three times. Least squares means and Tukey's HSD was used with a predetermined significance of P<0.05. The control was used as a reference and not included in statistical analyses. No animals or human subjects were used to generate data for this study.

III. RESULTS AND DISCUSSION

The variation in pH decline among treatments and control is shown in Figure 1. Arginine concentration impacted pH (P<0.05). Final pH was greater at 5000 (5.02) compared to 1000 ppm (4.78). L-Arginine HCL was confirmed to have a pH value of ~6.2. The Arg-HCl may have been converted to ammonia (NH_3) by bacterial arginine deiminase (ADI) enzymes contained in the *Pediococcus acidilactici* bacteria of the starter culture[2]. After 8-9 hours, the pH of all treatments was at or around 4.9. From hours 9-12 the pH of the treatments at concentrations of 3000, 4000, and 5000 ppm L-arginine appeared to increase. Conversely, the pH of the 1000 and 2000 ppm L-arginine treatments remained stable and did not increase. This indicates that the starter culture consumed available dextrose for the first 8-9 hours of fermentation and then for the final 3 hours, NH_3 as a by-product could have been generated causing the pH to increase for the 3000, 4000, and 5000 ppm L-arginine treatments. The mean pH of the nitrite control pH was 4.8, most similar to the 2000 and 3000 ppm Arg treated samples. Storage day did not impact (*P*>0.05) pH values for the nitrite control.

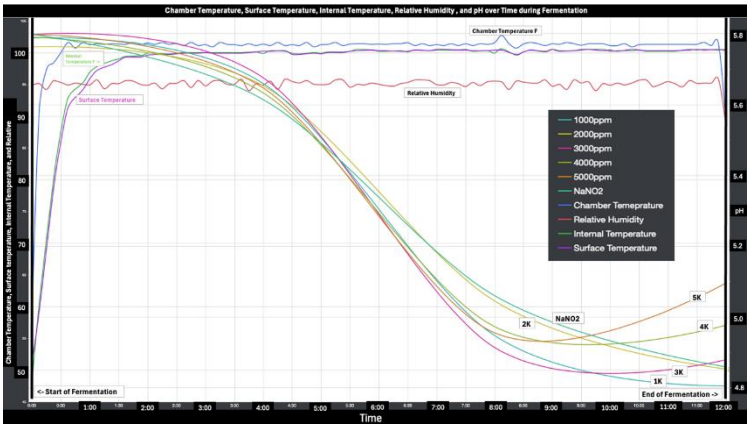


Figure 1. Fermentation pH decline.

Table 1. Least square means for main effects of arginine concentration and storage day residual nitrate (RNO3), residual nitrite (RNO2), nitric oxide heme (NO-Heme), total pigment, and curing efficiency of uncooked, fermented, and dried salami.

L-arginine Concentration (ppm)	RNO_3[1]	RNO_2[2]	NO-Heme[3]	Total Pigment[4]	Curing Efficiency[5]
1000	9.72[a]	3.23[a]	10.13[ab]	87.87[a]	12.22[a]
2000	8.25[a]	3.27[a]	9.49[ab]	86.66[a]	12.33[a]
3000	9.23[a]	3.32[a]	9.62[ab]	87.55[a]	12.52[a]
4000	7.70[a]	3.34[a]	10.98[a]	94.28[a]	13.29[a]
5000	8.56[a]	3.41[a]	7.69[b]	82.18[a]	12.46[a]
P-Value	0.76	0.47	<0.05	0.95	0.96
SEM[6]	1.16	0.07	0.75	9.87	1.10
Day	**RNO_3[1]**	**RNO_2[2]**	**NO-Heme[3]**	**Total Pigment[4]**	**Curing Efficiency[5]**
1	11.10[a]	4.80[a]	10.82[a]	134.55[a]	9.64[b]
14	10.63[a]	4.81[a]	8.22[b]	53.87[c]	15.80[a]
28	6.65[b]	2.03[b]	11.65[a]	98.10[b]	12.04[b]
56	6.39[b]	1.61[c]	7.62[b]	64.77[c]	12.78[ab]
P-Value	<0.01	<0.01	<0.01	<0.01	<0.01
SEM[6]	1.04	0.07	0.67	8.83	0.98
Nitrite Concentration (ppm)	**RNO_3[1]**	**RNO_2[2]**	**NO-Heme[3]**	**Total Pigment[4]**	**Curing Efficiency[5]**
156	54.65	4.08	44.05	84.45	52.87
SEM[6]	3.35	0.13	2.26	4.07	2.16
Day	**RNO_3[1]**	**RNO_2[2]**	**NO-Heme[3]**	**Total Pigment[4]**	**Curing Efficiency[5]**
1	54.50[a]	5.45[a]	40.77[a]	83.56[a]	49.74[a]
14	61.20[a]	5.09[a]	40.80[a]	75.23[a]	55.29[a]
28	63.42[a]	3.84[b]	42.43[a]	91.12[a]	47.60[a]
56	39.50[a]	1.96[a]	52.24[a]	87.89[a]	58.87[a]
P-Value	0.10	<0.01	0.27	0.56	0.27
SEM[6]	6.70	0.27	4.52	8.14	4.33

[1]RNO3 (ppm) = 14* (sample Area of NaNO3[mV.s] + 556.45)/692.43
[2]RNO2 (ppm) = 14* (sample Area of NaNO2[mV.s] + 1545.4)/952.11
[3]NO-heme = PPM Acid Heme of Nitrosohemochromagen
[4]Total Pigment = PPM of Hemoglobin in any its forms
[5]Curing efficiency - ppm nitrosohemachromagen/ppm of total pigment) × 100
[6]SEM: Standard error of the mean
[7]Nitrite Control: Used as a reference and not included in statistical analyses or concentration treatments
[abc]Means with the same superscript within a column are not significantly different (*P*<0.05)

Table 2. Least square means for main effects of arginine concentration and storage day on water soluble pigment levels expressed as percentages of deoxymyoglobin (DMB), oxymyoglobin (OMB), and metmyoglobin (MMB) of uncooked, fermented, and dried salami.

L-arginine Concentration (ppm)	%DMB[1]	%OMB[2]	%MMB[3]
1000	4.92[a]	66.96[a]	23.53[a]
2000	4.11[a]	70.36[a]	21.06[a]
3000	4.82[a]	76.27[a]	14.22[a]
4000	4.50[a]	78.92[a]	11.86[a]
5000	5.87[a]	78.44[a]	11.16[a]
P-Value	0.98	0.23	<0.05
SEM[4]	1.94	4.39	3.24
Day	**%DMB[1]**	**%OMB[2]**	**%MMB[3]**
1	1.32[a]	79.82[a]	14.15[a]
14	5.55[a]	69.30[a]	20.72[a]
28	5.28[a]	78.86[a]	10.80[a]
56	7.21[a]	68.79[a]	19.79[a]
P-Value	0.12	0.09	0.06
SEM[4]	1.74	3.93	2.90
Nitrite Control[5] Concentration (ppm)	**%DMB**	**%OMB**	**%MMB**
156	6.73	45.99	42.69
SEM[4]	3.82	6.75	4.47
Day	**%DMB**	**%OMB**	**%MMB**
1	-0.82[a]	56.24[a]	38.83[a]
14	8.81[a]	32.53[a]	54.77[a]
28	11.03[a]	40.96[a]	43.81[a]
56	7.90[a]	54.22[a]	33.36[a]
P-Value	0.71	0.57	0.41
SEM[4]	7.64	13.50	8.94

[1]Deoxymyoglobin expressed as a percentage of the total myoglobin present within sample
[2]Oxymyoglobin expressed as a percentage of the total myoglobin present within sample
[3]Metymyoglobin expressed as a percentage of the total myoglobin present within sample
[4]SEM: Standard error of the mean
[5]Nitrite Control: Used as a reference and not included in statistical analyses or concentration treatments
[ab]Means with the same superscript within a column are not significantly different (*P*<0.05)

Arg concentration did not impact (P>0.05) residual nitrate or nitrite values (Table 1). Residual nitrate decreased from day 14 to day 28 then stabilized through day 56. Residual nitrite decreased (P<0.05) beginning at storage day 14 through day 56. Control residual nitrate values were higher than Arg treated samples throughout storage, while residual nitrite values were similar between all Arg concentrations and the nitrite control. Arg concentration and day of storage exhibited a bimodal effect for NO-Heme values. Control NO-Heme values were 4X higher throughout storage compared to Arg treated samples. Arg concentration did not affect total pigment or curing efficiency (Table 1), while length of storage decreased total pigment values and curing efficiency, but had no effect on the nitrite control. Arg concentration impacted (P<0.05) metmyoglobin (MMB) percentages, however no mean differences were observed (Table 2). Nonetheless, as Arg concentration increased, MMB% tended to decrease, while the percentage of deoxymyoglobin (DMB) or oxymyoglobin (OMB) was not affected. While there were no statistical differences in OMB values, it was clear that as Arg concentration increased, OMB% increased and MMB% decreased. Arg treated samples exhibited higher OMB% and lower MMB% than the nitrite control with minimal differences in DMB. This can be partially attributed to the manner in which NO is bound to myoglobin in a conventional curing system in which NO is capable of binding with ferric metmyoglobin. In the eNOS system, O2 binds to DMB with greater affinity than NO resulting in greater levels of OMB as the NO generated by eNOS competes with O2 to bind with DMB[3]. This may result in greater color fading of Arg treated products.

IV. CONCLUSION

Results of this study indicated that higher Arg concentration impacts the rate of pH decline and ultimate pH, indicating that lower Arg concentrations should be considered. Residual nitrite values were similar in both treatment and control samples. However, control samples exhibited greater cured meat pigment color (NO-Heme) and curing efficiency. Therefore, additional investigation into manipulating the eNOS enzyme to enhance NO generation in post rigor skeletal muscle should be conducted. Challenges were also noted for the total pigment values as OMB% for Arg treated samples. This could impact color stability requiring the incorporation of antioxidants to stabilize color. The eNOS system is a potential alternative curing system for uncooked, fermented and dried sausage, however, enhancing NO generation, NO-Heme values and color stability would make it a more viable system.

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