NO-SYNTHASE ACTIVITY DEPENDING ON INCUBTION CONDITION AND ITS EFFECT ON MEAT COLOUR PARAMETERS

Marzena Zajac[1*], Krzysztof Zajac[2]

[1] Department of Animal Product Technology, Faculty of Food Technology, University of Agriculture in Krakow, Poland*Corresponding author email: marzena.zajac@urk.edu.pl

[2] Hare Scientific, Rudawa

I. INTRODUCTION

Nitric oxide (NO) synthase is an enzyme that is found in living cells, and it plays an important role in various physiological functions, including the regulation of blood pressure, the immune response, and neuronal signaling. Simplifying the reaction mechanism, it can be stated that NO synthase generates nitric oxide from arginine under certain conditions. Those conditions are different depending on the enzyme isoform: inducible, endothelial, or neuronal [1]. After slaughtering, when living muscle tissues are converted to meat, the conditions necessary for NO synthase activity are typically not present. It is important to note that while NO synthase is not used in the production of meat, there may be small amounts of NO present. The levels of NO produced through these mechanisms are much lower than what is produced through NO synthase activity in living cells. However, it is possible that, at artificially created conditions necessary for NO synthase activity, it could generate NO, which could be further used as a meat curing agent.This study aimed to determine the conditions for NO synthase activity in terms of obtaining the highest NO concentration to be further applied in meat systems and used for curing.

II. MATERIALS AND METHODS

L-Arginine solutions (0.1%) were prepared in water or HEPES buffer, enzyme NO-synthase (1U) (Sigma Aldrich, St Luis, USA) was added into 30 mL of all the solutions. Pork ground meat (5 g) was added to half of them. The solutions were incubated in 37°C or 20°C, for 1h or 2h. After the incubation time the samples were heated for 15 minutes in a water bath (95°C) to inactivate the enzyme. NO_2^- and NO_3^- ion contents were analysed using Griess method [2], pH was measured using pH meter (Elmetron Cp-505 electrode, Zabrze, Poland) Additionally, the colour parameters CIE*Lab* of meat samples was measured using Konica Minolta CM 3500d (Osaka, Japan). Two independent series of analyses were conducted. The results were subjected to ANOVA analysis, and the differences were tested using Tukey test at $P<0.05$.

III. RESULTS AND DISCUSSION

Inducible NO synthase (iNOS) is classified as EC 1.14.13.39, and is the only NOS isoform calcium and calmodulin independent at physiological conditions [3]. According to the results obtained in our studies, it is possible to generate NO from arginine in a water solution with iNOS added. The anticipated results were that conditions closest to physiological ones would be ideal to generate high concentrations of NO. Very low concentrations of nitrite ions were detected in solutions incubated with NO synthase. It was observed that more nitrate ions appeared in all the samples incubated at 20°C. Statistically significant differences were found only between buffer sample incubated for 2h at 37°C (no meat) and buffer sample incubated with meat, for 1h at 20°C. The presence of meat increased the concentration of both nitrite and nitrate ions. The pH values of pure solutions were 7.66 for HEPES and 7.35 for water arginine solutions. The pH drop was observed in water solutions containing meat regardless temperature and time conditions. It is possible that meat having lower pH (5.8) when mixed with solutions caused the decrease, which was especially noticable in samples with water in contrast to samples with buffer, which was able to block the pH drop. Another explanation is that, because of the basic character of arginine initially the solutions had higher pH, but when arginine was consumed by the enzyme, the pH dropped, also because the cytrulline, which has lowe pH.

Table 2. NO_2^- and NO_3^- ion concentration in solutions incubated at variable time/temperature conditions with or without meat (mean values ± standard errors)

Sample ID	Solution type	Temperature [°C]	Time [h]	pH	NO_2^- [µg/ml]	NO_3^- [µg/ml]
A	Buffer	37	1	7.53	0.03^{c} ± 0.01	0.10^{cd} ± 0.00
B	Water			7.59	0.03^{c} ± 0.02	0.07^{d} ± 0.01
C	Buffer		2	7.60	0.04^{c} ± 0.02	0.06^{d} ± 0.02
D	Water			7.50	0.07^{bc} ± 0.04	0.09^{cd} ± 0.02
E	Buffer	20	1	7.63	0.05 ± 0.03	0.28^{bcd} ± 0.10
F	Water			7.46	0.07^{bc} ± 0.04	0.20^{bcd} ± 0.07
G	Buffer		2	7.60	0.08^{bc} ± 0.04	0.28^{bcd} ± 0.13
H	Water			7.27	0.09^{bc} ± 0.05	0.33^{abcd} ± 0.20
	With meat					
A	Buffer	37	1	7.56	0.49^{ab} ± 0.02	0.47^{abc} ± 0.00
B	Water			7.29	0.08^{bc} ± 0.05	0.22^{bcd} ± 0.05
C	Buffer		2	7.51	0.50^{ab} ± 0.12	0.52^{ab} ± 0.21
D	Water			7.27	0.09^{bc} ± 0.05	0.28^{bcd} ± 0.10
E	Buffer	20	1	7.61	0.52^{a} ± 0.07	0.67^{a} ± 0.16
F	Water			7.21	0.14^{bc} ± 0.08	0.26^{bcd} ± 0.04
G	Buffer		2	7.43	0.35^{abc} ± 0.04	0.51^{ab} ± 0.14
H	Water			7.27	0.06^{c} ± 0.03	0.34^{abcd} ± 0.18

^{a,b,c,d} – different letters indicate statistically significant differences between means at $P<0.05$

The idea to introduce meat into solutions was based on a fact that NO limits the activity of NOS [4]. Meat was supposed to act as an NO-receiver constantly absorbing NO, which could further react with meat constituents, preferably myoglobin. Therefore, the colour of meat (cooked while disactivating NO synthase) was measured. All the samples incubated and cooked in a buffer solutions were significantly darker compared to all the samples in water. They had also lower *a** values except from sample G which was incubated at 20°C for 2h. Water samples showed significantly higher *b** values, except for sample G whish was comparable to the most of the water samples. Those outlying values obtained for sample G may be a result of relatively small meat sample and consequently lack of uniformity. Further studies need to be conducted to confirm those results. Unfortunately, *a** of meat sample cooked in arginine water solution was comparable to all the samples in the same solution with added enzyme regardless incubation conditions.

IV. CONCLUSION

Based on the nitrite and nitrate concentration obtained in the solutions it may be concluded that there was some NO synthase activity detected, especially in those with meat. Based on colour *a** parameter all meat samples in water solutions incubated at 20ºC were higher compared to the other samples. Unfortunately these results were comparable to the control sample of meat incubated in water solution containing arginine. Therefore, even if NO synthase was active, it did not affect meat colour.

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