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NITROSYLATING CAPABILITY OF SOME COAGULASE-NEGATIVE STAPHYLOCOCCUS (CNS) STRAINS IN A NITRITE FREE COOKED HAM MODEL SYSTEM

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I. INTRODUCTION

Despite nitrites have been added to meat products for years to improve the safety, enhance flavors and color stability and inhibit lipid oxidation [1], their elimination is a goal required by meat industry and consumers, since they are responsible for the formation of N-Nitroso compounds (NOCs) in processed meats and during their digestion, causing severe human diseases [2]. In recent years, alternative processes have been investigated to prepare healthier nitrite-free meat products, preserving their sensory quality. In this context, coagulase-negative staphylococcus (CNS) strains are promising candidates thanks to their ability to produce nitric oxide (NO) from nitrate and L-arginine naturally present in the meat through nitrate reductase (NR) and nitric oxide synthase (NOS) pathways, respectively [3]. The aim of this study was to investigate the capability of several CNS strains to develop the typical pink color, lowering the concentration of NOCs and residues of nitrites and nitrates in a nitrite-free cooked ham model system.

II. MATERIALS AND METHODS

Experimental design and processing method - *Semimembranosus* pig muscles were divided into four homogeneous batches and processed as reported in Table 1. Salt (1.8 g/100g), sodium ascorbate (0.05 g/100g), dextrose (0.05 g/100g) were also added to all trials. After tumbling, the muscles were sliced into 5 mm thick slices and distributed in 100 g portions in food-grade plastic bags. The SN, S and SY samples were incubated, then slices from all trials were cooked and cooled immediately on ice to stop any reaction (F_{70} =100'). Each treatment was replicated in duplicate.

Table 1- Processing conditions of each trial.

Trial	Ingredient (g per 100g of meat)	Brine injection (%)	Tumbling phase	Incubation phase	Cooking phase
C	Nitrite ion (0.0067)	25	Under vacuum, 720 rotations in 24 h	-	70°C x 100 min.
SN	mix of *Straphylococcus carnosus* (0.025) and nitrate-rich plant extract (0.227)	15	Under vacuum, 1882 rotations in 17 h	45°C x 2 h	70°C x 100 min.
S	mix of *Straphylococcus vitulinus* and *Straphylococcus xilosus* (0.025 g)	34	Pulsed vacuum, 3600 rotations in 48 h	40°C x 2 h	70°C x 100 min.
SY	mix of *Straphylococcus equorum* (0.075) and ingredient including yeast (1.0)	20	Under vacuum, 2986 rotations in 18.5 h	45°C x 2 h	70°C x 100 min.

Sampling and analysis – Representative samples were collected after tumbling, incubation and cooking phases, finely chopped with a knife, mixed to obtain a homogeneous sample, packed in plastic bags under vacuum and stored at -20 °C until analysis. *Nitrosylation yield of hematin (NHY)* – Hematin (H) and nitrosylated hematin (NH) were analyzed according to the method of Hornsey [4] with some modifications. Briefly, H and NH were extracted by homogenizing 5 g of the minced sample with 25 ml of mixture acetone:water: HCl (37%) = 80:18:2 (v/v/v) and acetone:water = 80:2 (v/v), respectively, with an Ultra Turrax homogenizer (T25, IKA, Königswinter, Germany) at 15,000 rpm for 1 min. After storage at 2 ± 2 °C in the dark for 1 h (H analysis) or for 5 min. (NH analysis), the sample was centrifuged for 10 min. at 10,000 × g at 4 °C. The supernatant was filtered through a glass microfiber filter (GF/A, Whatman, Little Chalfont, UK) in an amber bottle; then the absorbance was read at 640 nm (H analysis) and at 540 nm (NH analysis), against the reagent blank, by using a double beam spectrophotometer (Shimadzu, Milan, Italy) and quartz cuvettes, and it was multiplied by 680 and 290, respectively, to express the results as mg/kg of H and NH. NHY was calculated as ratio NH/H x 100. *N-nitroso compounds (NOCs)* – Total NOCs were quantified according to the method of Joosen et al. [5] with some modifications. Briefly, 50 mg of freeze-dried sample, weighted in a 3 ml syringe and diluted with 450 ml of water Milli-Q™, were incubated for 2 min. at room temperature with 100 µl of 50mM N-Ethylmaleimide (NEM) and 100 µl of 100 µM diethylenetriaminepentaacetic acid (DETP). Then, 500 µl of 5% (w/v) sulfamic acid solution was added to remove residual nitrite and after 3 min. the sample was injected into a purge vessel kept at 60 °C and filled with a standard tri-iodide reagent solution (38 mg of I_2 were added to a solution of 108 mg of KI in 1 ml water and dissolving it in 13,5 ml of glacial acetic acid). N-nitroso compounds different from nitrosothiols and nitrosyl iron (other NOCs) were quantified by first incubating with 100 µl of 10 mM $HgCl_2$ and 10 mM $K_3Fe(CN)_6$ solution for 2 min., and then following the procedure described above for total NOCs. The nitric oxide

(NO) released from the samples was detected by using an Eco Medics CLD 88 Exhalyzer (Eco Medics AG, Duernten, Switzerland) and quantified by a nitrite standard calibration curve in the range 10-100 μM. The results were expressed as nmol of NO per g of sample, taking into account sample and lyophilizate moisture in the calculation. *Nitrite and nitrate* - Residual nitrite and nitrate ions were determined by high-pressure ion chromatography [6]. A Thermo Scientific Dionex ICS-6000 system with an IonPac AS27 column and AG27 pre-column (30 °C) was used. Ten grams of meat samples were extracted in 100 ml water with 1 ml 1% NaOH (pH 8) in an ultrasonic bath for 45 min., then deproteinized by Vivaspin® 15 (5 kDa, 18 °C, 6000×g, 30 min). LOQs were 2 mg/kg for nitrites and 9 mg/kg for nitrates. *Statistical analysis* - Data were analyzed by using One-way variance analysis (ANOVA) of SPSS statistic software (version 22.0.0, IBM, NY, USA). The statistical differences were assessed using Duncan' test, *P-value* < 0.05 for the significance.

III. RESULTS AND DISCUSSION

Figure 1 shows the results of this study. Nitrosylation reactions occurred already in the tumbling phase, during which more than 90% of nitrite was consumed (trial C) and the CNS strains of S and SY trials, which exploited both the NR and NOS pathways, partially consumed the endogenous nitrate of meat and generated sufficient NO to achieve a NHY similar to the control, while controlling the total NOCS formation. The incubation phase was relevant in the SN trial, because *S. carnosus*, which mainly uses the NR pathway, showed variable performance during tumbling of replicates, as shown by the great variability of the NHY and residual nitrate values of the samples collected at the end of this phase. As to S and SY trials, small differences were observed for all parameters assessed in the samples collected after tumbling and after incubation.

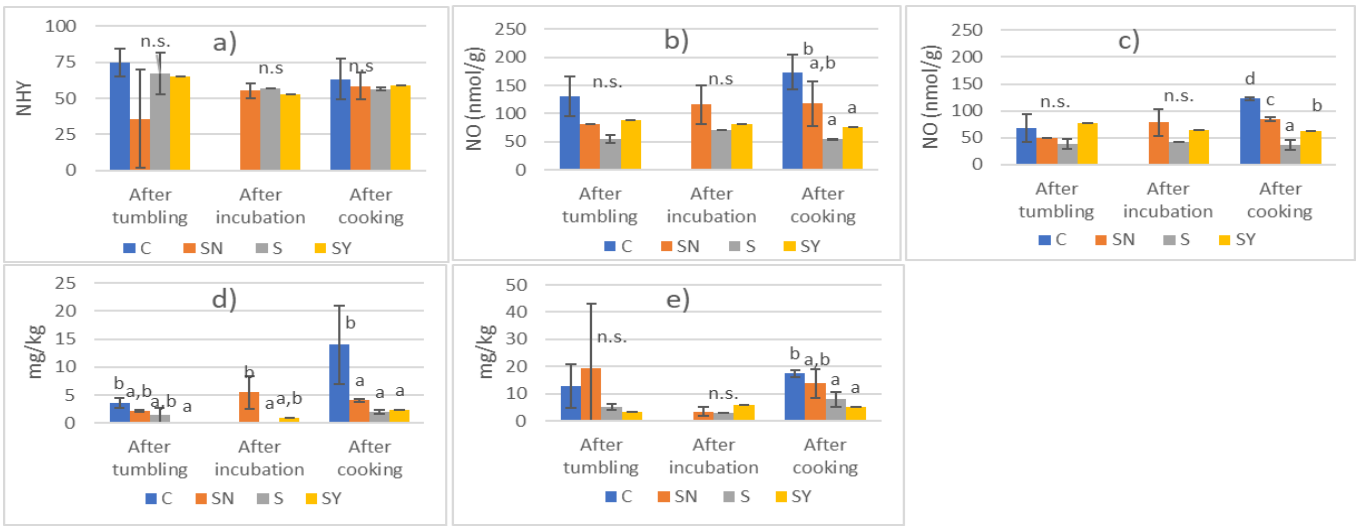


Figure 1. Nitrosylation yield of hematin (a), total NOCs (b), other NOCs (c), residues of nitrite (d) and nitrate ion (e) in samples collected after tumbling, incubation and cooking.

At the end of the processing, the profile of SN samples was very similar to the control in term of NHY, total NOCs and residual nitrate, while the S and SY samples had similar NHY, but significantly lower concentration of total and other NOCs and residual nitrite and nitrate.

IV. CONCLUSION

The combination of selected CNS strains with appropriate nitrate-free ingredients, along with process modifications, represents a promising strategy to produce nitrite-free cooked meat products with satisfactory sensory traits, mainly due to the development of the characteristic pink color, and to reduce the formation of N-nitroso compounds, which are potentially dangerous for human health.

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