

# CHANGES IN PROTEOLYSIS OF NITRITE-FREE ITALIAN-TYPE SALAMI MODIFIED IN FORMULATION AND PROCESSING

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## I. INTRODUCTION

Dry fermented sausages represent high-value commodities in many European countries. In their typical recipe nitrite acts as antimicrobial, antioxidant and colouring agent, exerting also a positive effect on product flavour. For some time already, health issues correlated with the use of nitrite in cured meats have been raised and the search for possible alternatives has been approached. So far, different strategies have been applied, like hurdle technology processing [1], the addition of natural antioxidants [2], and of nitric oxide from other sources [3]. In this frame, we performed a preliminary study to explore the effect of the changes made in salami formulation and process on proteolysis. The combined use of a protracted cold drying phase (at  $\leq 3$  °C) and the addition of natural antioxidants rich in polyphenols were the tools to balance the effect of nitrite removal on salami safety and quality [4]. An UPLC-MS characterization of peptides and free amino acids was performed to explore the impact of process and formulation changes on proteolysis in experimental salami.

## II. MATERIALS AND METHODS

Salami were produced in the pilot plant of the Meat Department of SSICA. The starting meat mixture was divided into four different formulations: CNO<sub>2</sub>, as “positive control”, added with sodium nitrite and sodium ascorbate; C0, as “negative control”, with no additives; SMA, added with sodium ascorbate and plant extracts as antioxidants. Commercial starter cultures were added to CNO<sub>2</sub> and SMA. CNO<sub>2</sub> underwent a conventional production process (CP), whereas C0 and SMA salami a modified process (MP), with a cold drying phase at  $\leq 3$ °C until a target pH and  $a_w$  decrease as a hurdle technology application [4].

For proteomics analysis, peptides and free amino acids (FAAs) were extracted according to Loffi et al. [5] and analysed with UPLC-MS. Peptides were analysed in full-scan mode, and semi-quantified by addition of an internal standard. FAAs were analysed in SIR-mode, after derivatization with a fluorophore, and quantified with a calibration curve of standard amino acids. After data processing, statistics was performed with One-Way ANOVA, employing Tukey’s test with  $p < 0.05$ .

## III. RESULTS AND DISCUSSION

The identified peptides showed a different distribution among salami groups, decreasing in the order SMA, C0, CNO<sub>2</sub>. SMA samples, manufactured with MP, were richer in medium-high molecular weight peptides and lower in FAAs. The more controlled proteolysis was ascribed to reduced endogenous proteases and microbial enzymatic activities in the MP conditions ( $\leq 3$ °C). On the other hand, CNO<sub>2</sub> samples, manufactured with a CP, showed a decrease in peptides and a significant increase of FAAs due to a more extended proteolysis, promoted by the standard temperature CP conditions. The trend of peptides and FAAs in the different formulations is reported in Figure 1.

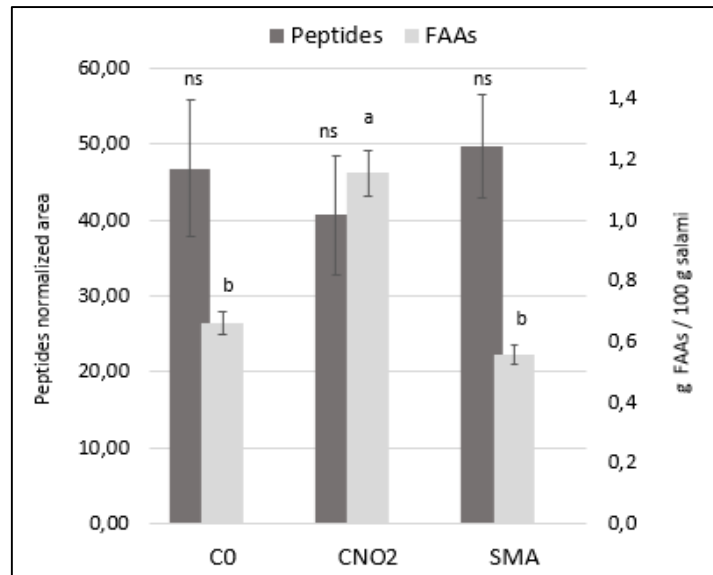


Figure 1. Peptides and FAAs trend in the different salami formulations. Significant differences are evidenced (ANOVA, Tukey's test,  $P < 0.05$ ).

#### IV. CONCLUSION

This preliminary study aimed to evaluate the impact of changes in formulations and processing in nitrite-free salami. The reduced temperature in MP, as a tool to inhibit pathogen growth, led to a modified proteolysis in nitrite-free salami, which may turn into a different sensory profile. Further strategies will be explored to obtain safe nitrite-free salami with a sensory profile comparable to the traditional ones.

#### ACKNOWLEDGEMENTS

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