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STAPHYLOCOCCI AND ZNPP-CONTAINING LIVER EXTRACT AS NITRITE-FREE ALTERNATIVES ENHANCING THE RED COLOUR OF DRY-FERMENTED MEATS

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I. INTRODUCTION

Nitrates and nitrites are widely used in the meat industry. However, they are involved in the production of genotoxic substances, and meat producers are moving toward clean-label products. The objective of this study was to investigate the potential of alternatives to pure nitrate and nitrite sources to improve the colour of dry fermented meats through selected staphylococci applied as starter culture (*Staphylococcus equorum* L33 or *Staphylococcus saprophyticus* L49 showing a nitric-oxide synthase activity (NOS)), a porcine liver extract having a high zinc protoporphyrin (ZnPP) content, and a polyphenol-rich ingredient, alone or in combination with the cultures and ZnPP-rich liver extract.

II. MATERIALS AND METHODS

Each experimental treatment utilised 1 kg of minced pork meat, seasoned with sodium chloride (2.5%) and dextrose (0.4%) to elaborate model dry-fermented sausages. The study included the positive control consisting in a nitrite-cured meat batter prepared with sodium ascorbate (0.05%) and sodium nitrite (150 mg/kg) (CTRL+), the negative control (CTRL-) consisting solely of meat batter with sodium ascorbate (0.05%), the latter inoculated with *S. equorum* L33 (L33), the same inoculated with *S. saprophyticus* L49 (L49), the same involving the addition of ZnPP extract (ZnPPEx), as well as their combination with the addition of 1% of the polyphenol-rich ingredient NATPRE T-10 CUR HT (Prosur, Spain) (CTRL-/NATPRE, L33/NATPRE, L49/NATPRE, and ZnPPEx/NATPRE, respectively). *S. equorum* L33 and *S. saprophyticus* L49 were inoculated at a level of approximately 6 log CFU/g, while the treatments involving the addition of ZnPP extract (prepared as described by Llauger et al. [1]) were formulated to contain 40 mg ZnPP/kg. For each treatment, meat batter portions weighing 83 g were transferred into custom-made bags made of a Tublin® 05 film. Bags were sealed by vacuum packaging, and then ripened in a controlled environment at 8 °C. Product analyses (in triplicate) involved the investigation of pH, moisture content, water activity, technological microbiota counts, amount of hemin, nitrosyl-heme and ZnPP, UV-Vis spectra and urea-PAGE electrophoresis of aqueous extracts, and colour attributes were performed immediately after preparation and subsequently at 7, 14, and 21 days of ripening.

The experiment was fully replicated at two different times using two batches of meat. All 216 samples deriving from the two experimental trials (3 samples per treatment × 9 treatments × 4 ripening periods × 2 replicates) were included in the statistical analysis.

III. RESULTS AND DISCUSSION

All the elaborated model dry-fermented sausages resulted in low-acid products, mainly because of the low amount of dextrose added to the formulation, the low process temperature, and the low levels of endogenous lactic acid bacteria in pork. The model sausages were characterised by a moisture content around 61% at the beginning of the ripening process, and around 37% after 21 days. All treatments showed final a_w-values lower than 0.91, values described to inhibit the growth of relevant pathogens [2].

CTRL+ treatment was featured by high levels of hemin and nitrosyl-heme. Similarly, the addition of NATPRE consistently determined higher values of both hemin and nitrosyl-heme when compared to the corresponding treatments without this ingredient, due to its residual amount in nitrates and nitrites. On the other hand, the hemin and nitrosyl-heme contents of the L33, L49, and ZnPP-containing treatments were similar to that of the CTRL- throughout the ripening, indicating minimal formation of nitrosyl-heme. Regarding ZnPP content, the CTRL+ and CTRL- treatments recorded the lowest concentrations, whereas ZnPPEx and ZnPPEx/NATPRE samples showed the highest levels.

In almost all treatments, UV-Vis spectra of the aqueous extracts showed the maximum peak of absorbance at around 410 nm, the characteristic Soret band of porphyrins. In the samples containing ZnPPEx and additional secondary peak at 420 nm indicating the presence of ZnPP [3] was also observed. These findings were in agreement with the reported ZnPP content and suggested that at least a part of the ZnPP remained soluble.

These outcomes were in accordance with the urea-PAGE gel electrophoresis results. In fact, two intense fluorescent bands were visible in the ZnPP-containing treatments, suggesting that at least part of the ZnPP content was bound to soluble proteins, likely hemoglobin. Lower-intensity fluorescent bands with the same size were also observed in the other treatments, consistent with the lower ZnPP content observed by HPLC.
Regarding colour attributes, the CTRL- treatment exhibited significantly lower redness values than those of the other eight treatments at the investigated ripening times, while all the NATPRE-containing sausages showed better redness values than the CTRL-, but still lower than CTRL+. On the other hand, model dry-fermented sausages elaborated with *S. equorum* L33 and *S. saprophyticus* L49 showed the characteristic red colour of nitrite-cured meat, as previously demonstrated by Premi et al. [4]. In fact, L33 treatment featured the highest *a**-values, equal to 7.5, 9.5, 9.9, and 10.5 after 0, 7, 14, and 21 days of ripening, respectively. Similarly, L49 showed an initial red pigmentation value of 7.6, which increased to 9.1, 10.0, and 10.1 over the same ripening periods. Given the minimal formation of nitrosyl-heme in L33 and L49 treatments, other mechanisms, such as the catalase activity, reducing power, oxygen consumption, and proteolysis could also be involved in the colour enhancement of the CNS-containing treatments. In fact, the redness of L33 and L49 treatments was always featured by the highest statistical significance, reaching the maximum *a**-values after 21 days of ripening. Interestingly, ZnPPEx treatment exhibited the pinkish-red hue typical of traditionally cured meat, showing *a**-values equal to 7.0, 8.6, 9.9, and 10.1 at the four analysed ripening times. The intense redness in ZnPPEx sample revealed that the porcine liver extract having a high ZnPP content could improve the colour of dry-fermented sausages without the addition of nitrite. Therefore, these results encourage its application as a colouring ingredient in meat-based products.

IV. CONCLUSION

Red colour appearance of dry-fermented sausages can be improved by the addition of selected coagulase-negative staphylococci showing NOS activity, porcine liver extract having a high ZnPP content, and/or the addition of the NATPRE T-10 CUR HT ingredient. While the application of a ZnPP extract is a winning strategy in the replacement of synthetic additives owing to this pigment intrinsic colour, the NOS activity performed by the selected bacteria may be part of a more complex mechanism that ultimately results in the improvement of redness. Further research is needed to evaluate the impact of bacteria and ZnPP-rich liver extract on the sensory profile of the final products.

KEYWORDS

coagulase-negative staphylococci; fermented sausages; nitric oxide synthase; nitrosyl-heme; zinc protoporphyrin

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