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THE FUTURE IS NOW: THE USE OF *THYMUS CITRIODORUS* AND *SALVIA ELEGANS* AS ALTERNATIVES TO NITRITE IN MEAT PRODUCTS

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I. INTRODUCTION

The preservation of meat through curing is associated with the use of salt and nitrites. These nitrite curing salts have technological properties associated with oxidation inhibition, cured-meat color and aroma formation [1]. However, nitrites are precursors of N-nitrosamines, a group of compounds with potential carcinogenic effects. In 2015, processed meats were classified as carcinogenic to humans for colorectal cancer [2]. Since then, several researchers have been looking for nitrite alternatives for cured meat products such as the use of nitrate-rich aromatic plants, but still, no obvious solution was found [3]. From October 2025, ten years after the International Agency for Research on Cancer (IARC) classification, the amount of nitrite allowed in meat products will be reduced, according to Commission Regulation (EU) 2023/2108 [4]. Now, it is imperative not only to find a solution for meat products, but also to understand the consequences of lowering their ingoing amount of nitrite. The aim of this study is to evaluate: 1) the effect of reduced ingoing amounts of nitrite and 2) the effect of the use of *Thymus citriodorus* (thyme) or *Salvia elegans* (sage) infusions with and without a *Staphylococcus equorum* starter on the safety, quality and sensory acceptance of a cured meat product (bacon).

II. MATERIALS AND METHODS

Seven batches of bacon (one batch per formulation) were produced on three different working days. For each working day 7 pork bellies were manually deboned and adjusted to 5 kg. Each pork belly was randomly attributed to formulation and injected with 10% cold brine (w/w; 5ºC). The formulas were: F1 - without $NaNO_2$; F2 - 150 mg $NaNO_2$/kg; F3 - 80 mg $NaNO_2$/kg; F4 - thyme infusion; F5 - thyme infusion with starter; F6 - sage infusion; and F7 - sage infusion with starter. The water of the brine was replaced by the thyme or sage infusions (5% dry matter in hot water, 10 min; cooling to 20ºC). The injected pieces were stored (5 days, 3ºC). After brining period, the pieces were dried and smoked in a heated chamber to reach 65 ºC internal temperature during 15 min. After the smoking cycle, bacons were maintained at 10 ºC for 36 h. Lactic acid bacteria (LAB), coagulase-negative staphylococci (CNS), and *Enterobacteriaceae* were counted according to ISO Standards, on raw meat (M), after injection (AI), after brining period (AB), and in final product (FP). Detection of *Salmonella* sp. and *Listeria monocytogenes* was performed on FP. Residual nitrate and nitrite, A_w, pH, and color measurement using *L*a*b** color space with a Konica Minolta CR-400/410, illuminant D65 were determined on AI, AB, and FP. Sodium chloride was determined on FP. TBARS were assessed on M and FP. The model of statistical analysis considered the effects of formulation, timepoint and interaction. The batch was considered the experimental unit. The three different working days were considered as blocks. The GLM procedure of SAS® was used to analyze the variance of the various response variables. Significant differences were considered when $p<0.05$. Two focus groups (GF1 - 10 consumers, 7 females; GF2 - 9 consumers, 5 females) were carried out to evaluate the effect of different formulations on bacon sensory attributes. The discussion was guided by a researcher with experience in this technique. The samples were presented to the group and the discussion was qualitative. Participants were asked to identify the attributes they identified in each sample, particularly regarding color and aroma, and to evaluate the overall appreciation, also using qualitative open questions.

III. RESULTS AND DISCUSSION

The absence of *L. monocytogenes* and *Salmonella* spp. in 25 g of all final products was confirmed. Nor the formulation nor the time had any effect ($P = 0.82$) on LAB counts. *Enterobacteriaceae* counts were influenced by time ($P = 0.02$), reaching 3.07 log cfu/g after brining, with an expected significant decrease after the thermal treatment (FP = 2.14 log cfu/g). The interaction between formulation and time was significant ($P < 0.01$) for CNS counts. During the processing steps, CNS counts were higher ($P < 0.05$) in the inoculated products (F5 and F7),

varying between 5.77 and 6.14 log cfu/g. Nevertheless, all final products presented no differences regarding CNS counts, which were below 3.26 log cfu/g. The effect of different formulations had no impact (P = 0.82) on the NaCl content of final product, being 1.9% in average. The variables pH, A_w, and L^* were also not affected (P > 0.05) by the different bacon formulas, nor the interaction between formulation and time, meaning that only the different stages of processing affected (P < 0.01) these parameters. On the contrary, color parameters a^* and b^* were influenced (P < 0.01) by the formula/time interaction. During the processing independently of formulations, the A_w of samples significantly decreased (P < 0.05), from 0.98 after the injection of the brine to 0.97 on final product. The pH decreased from 5.83 after injection to 5.75 after brining. Then pH increased, reaching 5.98 on the final products. Products color showed a significant increase (P < 0.05) in L* overtime, especially from after brining step (L* = 45.70) to final product (L* = 65.51). Regarding a^* values, products formulated without nitrite (F1, F4, F5, F6, and F7) presented a decreasing tendency overtime, from values ranging between 14.74 (F1) and 20.87 (F6) after injection to a^* values ranging between 5.80 (F5) and 6.67 (F1) on final product. Once an increase in a^* values generally represent an increase on redness, the observed decrease might be attributed to the oxidation of myoglobin to metmyoglobin followed by its denaturation during thermal treatment [1]. Products formulated with nitrite (F2 and F3) presented lower a^* values variation, with no significant differences (P < 0.05) between different ingoing amounts of nitrite (F2 - 150 mg $NaNO_2$/kg; F3 - 80 mg $NaNO_2$/kg) nor different processing steps. Due to the putative formation of nitrosohemochromogen, the final product formulated with nitrite had pinkish color, presenting a^* values between 13.72 (F3) and 13.11 (F2) while products formulated without nitrite presented a^* values significantly (P < 0.05) lower, below 6.67 (F1). Results from b^* values ranged from 6.20 (F1 after injection) to 11.05 (F6 after injection). These differences in color were pointed out by some participants of the focus groups, which considered products formulated with infusions less appealing than those formulated with nitrite. The effect of formulation was significant (P <0.01) for residual nitrite content with F2 and F3 bacon presenting higher residual levels of 23.75 and 10.15 mg $NaNO_2$/kg, respectively. Still, the residual levels found are in accordance with the new maximum of 45 mg NO_2/kg [4]. Products formulated with infusions (F4, F5, F6, and F7) presented much lower (P < 0.05) residual nitrite content, below 0.41 mg $NaNO_2$/kg. Residual nitrate content was also influenced (P < 0.05) by the bacon formula, despite that all contents were inferior to 4.67 mg $NaNO_3$/kg. The effect of the interaction formula/time was highly significant (P < 0.001) on TBARS, ranging from 0.08 to 0.19 mg MDA/kg on raw meat and from 0.19 to 1.44 mg MDA/kg on final product. Interestingly, bacon formulated without nitrite, nor infusions, (F1 = 1.44 mg MDA/kg) presented MDA values three times higher than all the remaining products (< 0.37 mg MDA/kg) which did not present differences between them. These results emphasize the antioxidant capacity of nitrites [1], but also the benefits of the bioactive compounds, such as phenolic compounds, present in plant infusions [3]. Further studies will be done to assess the effect of these infusion and low nitrite ingoing amount on the shelf life of the products.

CONCLUSION

Reducing the ingoing amount of nitrite from 150 mg $NaNO_2$/kg to 80 mg $NaNO_2$/kg did not impact the overall quality of bacon. Products produced with aromatic plant infusions without starter showed *Enterobacteriaceae*, CNS and LAB counts similar to those formulated with nitrite. The presence of starter in the formulation with infusion of *T. citriodorus* and *S. elegans* did not have any positive effect on final product color. In addition, the use of infusions resulted in bacon with very low residual nitrite and TBARS values, achieving the objective of reducing nitrite while improving antioxidant capacity. *Thymus citriodorus* and *Salvia elegans* infusions might be the solution for eliminating nitrites in meat products, requiring further processing optimization. Consumer tests will allow to understand if these products are appealing and able to launch in the market.

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