MULTIRESISTANT *STAPHYLOCOCCUS AUREUS* VS. PH, LIQUID SMOKE, BACTERIOCINS, AND ESSENTIAL OILS

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I. INTRODUCTION

In recent years the increased prevalence of multiresistant *S. aureus* associated with the food chain, in particular meat products, has become a significant public health concern [1]. Nevertheless, many processing factors can limit the growth and reduce the bacteria load while manufacturing traditional meat products. Two important processing steps that can inhibit microorganisms' growth are fermentation and smoking/drying [2]. During fermentation, the pH is lowered due to the development of lactic acid bacteria (LAB); this lower pH can negatively affect other bacteria, namely *S. aureus*. Some LAB can also impact the inhibition of pathogens by producing bacteriocins [3]. Smoking is also important to control pathogens, due to the antimicrobial effects of smoke composition [1]. Other strategies to control pathogens have been reported, including the use of essential oils [5]. This work investigated the antimicrobial effects of different approaches based on pH, liquid smoke, bacteriocins, and essential oils on multiresistant *Staphylococcus aureus* recovered from a meat food chain.

II. MATERIALS AND METHODS

Five strains of methicillin-resistant *S. aureus* had their antimicrobial profile studied, utilizing the disk diffusion method. The ability of the strains to adapt to the pH and liquid smoke was tested by inoculating them at a concentration of 10^6 bacteria in Brain Heart Infusion Broth (BHI). The pH values tested were: 3, 4.5, 5.5 and 7.5. The liquid smoke concentrations were: 4%, 2%, 1%, 0.5%, 0.25%, 0.125% and 0.625%. The bacteria growth at 37ºC was evaluated in both experiments at 0h, 6h, 12h and 24h by turbidimetry and enumeration of colony-forming units (cfu/ml).To assess the effect of semi-purified bacteriocins (several enterocin, Mundticin-like, nisin, gallidermin) and essential oils (Dill, Pine, Anise, Juniper berry, Chamomile, Peppermint, Thyme, Sage, Coriander, Cumin, Fennel and Oregano) the method performed was the "Agar Spot Test". The five strains *of S. aureus* and one sensitive strain for bacteriocins (*E. avium* EA5) were inoculated when they reached an absorbance approximately of 0.8 and then incubated for 24h at 37ºC. For the essential oils, the inhibition halos diameter was measured and for bacteriocins arbitrary units (AU/ml) were determined.

III. RESULTS AND DISCUSSION

The five *S. aureus* strains were found to be resistant to gentamycin, oxacillin, cefoxitin, clindamycin and tetracycline. These multiresistant strains were then tested for their ability to grow under different pH levels at 37ºC. None of the strains was affected by the pH of 7.5 and pH 5.5. At pH 4.5 most strains demonstrated a decrease in log cfu/ml values over 24 h, apart from the strain ZP25 which presented an increased log value between 12h and 24h, indicating that the strain was able to adapt to the pH stress (Figure 1). All strains were affected by pH 3, decreasing the log cfu/ml values to the detection limit of 1 log cfu/ml after 12 h. The pH of most traditional fermented meat products could inhibit most of these multiresistant strains since meat products' pH is usually around 4.5. However, it is still a concern that some can persist under low pH. The liquid smoke assay showed that all strains were resistant, meaning that all strains could grow under the conditions indicated by the

manufacturer (0,5%) (Figure 2). Liquid smoke percentages of 1% and higher showed the ability to decrease the presence of the *S. aureus* strains. Nonetheless, while liquid smoke has been in past years used as a substitute for the traditional process, it is still different. The concentrations of smoke in the conventional smoking process can be variable depending on the location of the strains in the food product, and the time of smoking is important also. To completely understand the capacity of these strains to maintain themselves on the product during smoking, further investigation should also be performed because smoking is done with drying. The resistance capacity of these strains was also extended to the twelve bacteriocins tested. All five *S. aureus* were completely resistant to all bacteriocins tested, except nisin and gallidermin. Gallidermin was more effective than nisin, with the strains ZP25 and ZAP5 being more susceptible (12800 AU/ml). This resistance to bacteriocins is not uncommon and has been detected by several other studies. All 12 essential oils were able to inhibit growth, apart from pine and chamomile essential oils. The essential oils that demonstrated a higher capacity to affect these staphylococci were oregano, coriander, and thyme. Other studies have detected this same antimicrobial capacity of essential oils, nevertheless, there is still a need for more studies to determine the dose needed for maximum efficacy and how to apply these compounds, so they are not affected by food pH and storage temperature.

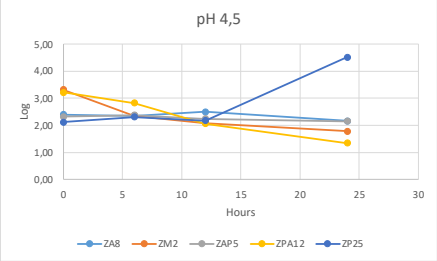

Figure 1. Strains growth with a pH of 4,5.

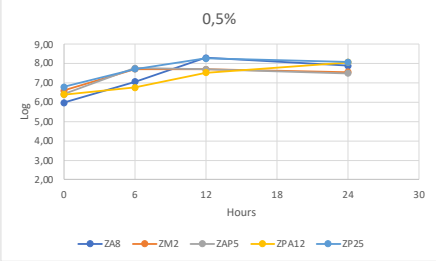

Figure 2. Stains growth with 0.5% of liquid smoke.

IV. CONCLUSION

The pH was the main factor of meat fermented and smoked sausages influencing the population load of multiresistant *S. aureus* strains. All other strategies tested are ineffective in reducing and controlling these strains, except for using nisin, gallidermin, and most of the essential oils tested. However, while bacteriocins could impact modulating all meat sausage microbiota, adding essential oils can modify the sensory attributes of meat products, making them undesirable to the consumer; these subjects should be investigated further.

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