VARIATION IN NITRATE REDUCTASE OF STAPHYLOCOCCI STRAINS AND OPTIMAL COLOR IN LOW-NITRITE CURED MEAT PRODUCTS

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I. INTRODUCTION

The application of additives nitrite (E249/E250) and/or nitrate (E251/E252) into cured meat products has been topic of controversy for decades [1]. Beginning of 2023 a 5-year plan for revision and gradual reduction of legal limits for nitrite and nitrate in various cured meat products has been announced for France and a revision of permitted levels is also on-going at EU level. An immediate reduction to 80-100 ppm is effective in France with a gradual decreasing level in the years to come. Such a reduction in in-going amount of nitrite and nitrate will present challenges to maintain similar product quality regarding colour and taste/flavour and certainly also regarding microbiological safety. The ability and efficiency of staphylococci strains to impact pools available of nitrite and/or nitrate in cured meat product is well-known (2), and it can prove even more critical when permitted levels of added nitrite (or nitrate) is reduced significantly, which means that utilization and regeneration of for instance nitrate becomes vital to ensure wished quality and sensory properties.

II. MATERIALS AND METHODS

Three experimental groups of selected staphylococci strains; CH1 (*S. vitulinus)*, CH2 (*S. xylosus*), and CH4 (*S. carnosus*) were initially screened in duplicate for growth rate in TSB under anaerobic incubation at the following temperatures 10 °C, 15 °C, 20 °C, 23 °C, 26 °C, 30 °C and 37 °C. Growth rate was monitored as optical density at 600 nm (OD_{600nm}) after 24 hours incubation on duplicates at all temperatures yielding a total of 42 observations. The staphylococci strains were also screened for nitrate reductase activity as described by Casabuni et al. [2], in which samples of YT broth added 250 ppm KNO_3 were inoculated with 100ml of overnight culture. Assessment of nitrate activity was conducted after anaerobic incubation for 0, 2, 4, 6 or 8 hours at 37°C in duplicates giving a total of 24 observations. Assay measured nitrite by centrifuging 1 ml of culture sample to remove cells, and then adding 100 µl supernatant to 2 ml distilled H_2O followed by 500 µl of Griess reagent. Mixture was mixed on vortex for 1 minute and allowed to react for 10 min at room temperature, before nitrite concentration was determined as absorbance at 540 nm.

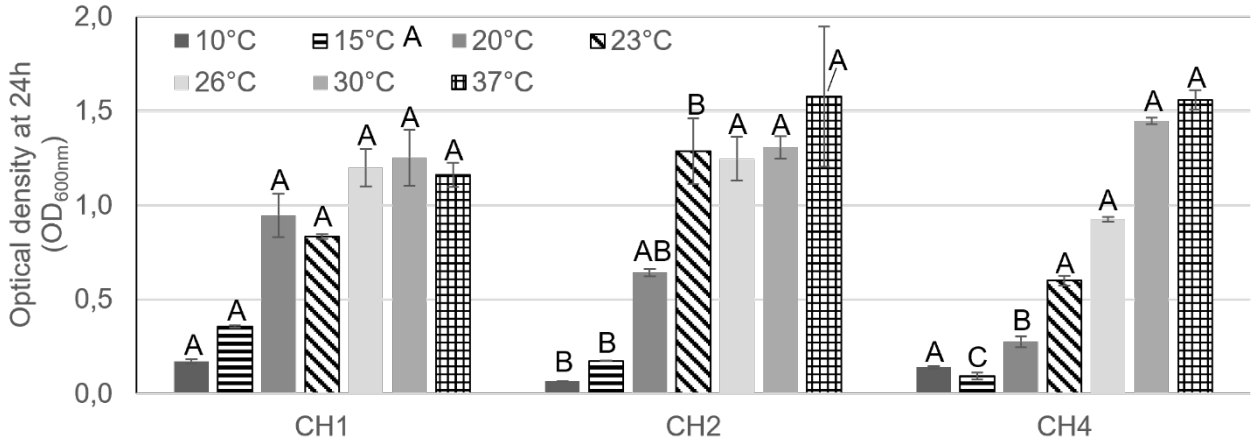

Figure 1. Growth after 24h of 3 staphylococci strains under anaerobic incubation at 7 temperatures. Means for each temperature compared by Tukey test and letters indicate significant different values (P<0.05).

III. RESULTS AND DISCUSSION

Ability to grow at varying incubation temperatures in Fig. 1 shows some variations for the 3 staphylococci strains, where the strain CH1 exhibits significant better ability to grow at temperatures up to 20 °C compared to strains CH2 and CH4, respectively ($P<0.05$). Strain CH2 is growing best at 23 °C ($P<0.05$), while all 3 strains show equal growth rates at all temperatures when incubated at 26 °C, 30 °C and 37 °C. Fig. 2 shows that CH1 strain is by far most active in nitrate reductase, i.e., with fastest and highest response in relative activity. The fast response in expression the enzyme means that already after 2 hours it is detected and at 4-hour enzyme activity almost reaches a maximum level. In contrast, the CH4 strain shows no sign of converting nitrate to nitrite as activity is always close to zero (it is however, possible that a nitrite reductase is active which then continuously converts product nitrite into other metabolites leading to this false negative result). The strain CH2 exhibits no enzyme activity during initial 4 hours, but at 6 and 8 hours it reaches its maximal level. The ability to response fast and effectively reduces nitrate into nitrite is crucial when considering lowered in-going levels of nitrite. In meat curing there are several known steps leading to nitrate, i.e., initial nitrite oxidation of oxymyoglobin, disproportion of N_2O_3 and discoloration of nitrosylmyoglobin, and with these reactions in mind the regeneration or reactivation of that nitrate pool is critical.

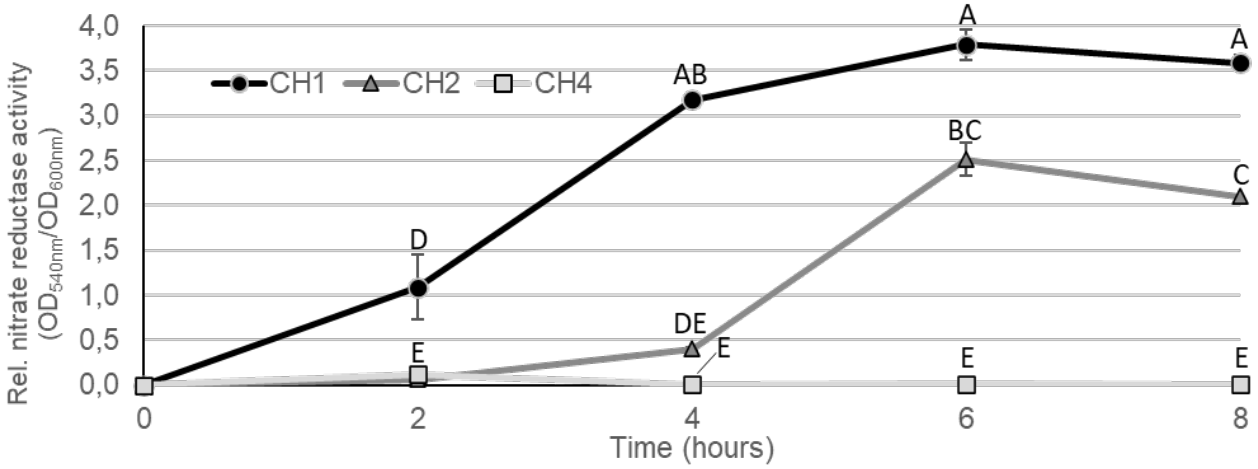

Figure 2. Relative nitrate reductase activity of 3 staphylococci strains at 37°C over time. Nitrate reductase determined as nitrite level measured at 540 nm via reaction with Griess reagent, while growth estimate is monitored as optical density at 600nm. Letters indicate significant different values by Tukey test ($P<0.05$).

IV. CONCLUSION

Results herein show that nitrate reductase activity in staphylococci is highly strain dependent and not necessarily directly related to growth rates. The findings will be further studied in model system assays, in which varying combinations of nitrite and nitrate at levels ranging from very low 15 ppm up to new legal limits at 75 ppm will be investigated with respect to impact of staphylococci in controlling and optimizing balance between these species in favour of quality and sensory aspect of the meat product.

REFERENCES

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