INFLUENCE OF NITRATE REDUCTASE ACTIVITY OF *STAPHYLOCOCCUS CARNOSUS* STRAINS ON COLOR STABILITY OF SLICED CURED RAW HAM PACKED UNDER DIFFERENT CONDITIONS

Kurt Herrmann[1], Ramona Bosse[1], Julia Freese[1], Monika Gibis[1], Anne Müller[2] and Jochen Weiss[1]

[1]Department of Food Physics and Meat Science, [2]Department of Food Microbiology and Hygiene, Institute of Food Science and Biotechnology, University of Hohenheim, Garbenstrasse 21/25, 70599 Stuttgart, Germany

The influence of nitrate reductase activity of *Staphylococcus carnosus* starter cultures on the color stability of sliced cured raw hams was investigated during 12 days of storage under 4 different packaging conditions. More precisely hams were stored under vacuum (dark and light) and under residual air (dark and light). Therefore, brine either with nitrite or nitrate was injected with or without *S. carnosus* strains to produce raw hams. Microbiological, chemical and sensory analyses were carried out during 3 weeks of processing and 12 days of storage to evaluate the effect of the applied combinations. Raw ham produced with *S. carnosus* LTH 7036 (high nitrate reductase activity) showed the highest decrease in nitrate content compared to hams cured with nitrate and without starter culture or with *S. carnosus* LTH 3838 (low nitrate reductase activity). The strains with high nitrate reductase activity improved the color stability of the raw ham slices during storage. This effect was explained by the improved reduction of nitrate to nitrite that stabilizes the color pigments in cured raw ham. Therefore, the nitrate reductase activity plays an important role when selecting starter cultures for the production of raw cured ham.

Key Words: raw ham curing, storage, specific enzyme activities, starter cultures

I. INTRODUCTION

Meat curing is an old and traditional technique to preserve meat from spoilage [1, 2]. Nowadays one of the main objectives in raw ham processing is to improve and stabilize the typical red color [1, 2]. That becomes more and more important because most of the products get sold as slices in self service packages.

Nitrite is the agent that is responsible for the formation of nitric oxide that reacts with the meat pigment myoglobin to the typical reddish nitrosylmyoglobin [2]. When adding nitrite to meat products, it is partly oxidized to nitrate and has to be reduced to nitrite by the bacterial nitrate reductase. Moreover, nitrite added directly or produced by microorganisms with nitrate reductase activity has more benefits in raw meat products including the suppression of growth of anaerobic bacteria such as *Clostridium botulinum*, flavor development, and its antioxidative activity [1, 2]. In Europe the usage of nitrite and nitrate is strictly regulated by the government. The maximum amount of sodium nitrite for raw ham is 50 mg/kg and potassium nitrate is 250 mg/kg in the final product (Regulation (EC) No. 1333/2008). Starter cultures with nitrate reductase activity such as staphylococci could be used to control the fermentation and reduction of nitrate even when only nitrite is used due to the disproportionation of nitrite to NO and nitrate [3]. Therefore the nitrate reductase activity is an important factor for the selection of starter cultures used for raw ham fermentation.

The aim of this study was the investigation of the influence of the nitrate reductase activity of selected *Staphylococcus carnosus* strains on the formation of nitrate and nitrite and the color stability of sliced raw hams under different packaging conditions.

II. MATERIALS AND METHODS

II.1 Selection and Preparation of Starter Cultures

S. carnosus LTH 3838 was chosen because of its low nitrate reductase activity and its high proteolytic activity and *S. carnosus* LTH 7036 was

used due to its high nitrate reductase activity [4]. The bacterial strains were grown (for 24h at 37°C in nutrient broth) and afterwards concentrated by centrifugation to a bacterial cell concentration of approx. 10^{11} cfu/mL.

II.2 Raw Ham Production

Fresh pork loin (pH: 5.46 ± 0.05; *Musculus longissimus dorsi*) was obtained from a local wholesaler (MEGA, Germany). For all four batches 30 L brine was made with 10% curing salt and done in duplicate. Nitrate curing salt was produced by using 13.18 g KNO_3 (Gewürzmüller, Germany) per kg NaCl. For batch 1 (nitrate control) the brine contained just nitrate curing salt. The second batch (nitrite control) was injected with brine containing a commercial 0.9% sodium nitrite curing salt (Südsalz, Germany). The brine with *S. carnosus* LTH 7036 (batch 3) was produced by using nitrate curing salt and 1 mL bacterial suspension to reach a total staphylococcal count of 10^6 cfu/mL in the brine. Batch 4 was injected with brine containing *S. carnosus* LTH 3838 and nitrate curing salt. All hams were injected using a multi needle injector 105 MC2 R (Günther, Germany) with the following settings: 105 needles (2 mm diameter, 2 x 0.8 mm hole size), 6 bar injection pressure, to get an injection weight of 7 - 8% of fresh meat weight. After injection, the hams where rubbed with the equivalent salt to a final salt content of 40 g per kg fresh meat. Dry curing was done in plastic boxes for 7 days at 5 °C. Drying and mild smoking (at 24 °C) took place in the climatic chamber Air Master UK-1800 BE (Reich, Germany) for 7 days with temperatures of 10 to 15 °C with a relative humidity gradient from 85 to 75%. Afterwards hams were stored at 15 °C at 75% rel. humidity till they reached a final weight loss of 24.2% (± 2.05%). For each storage condition three ham slices with a thickness of 50 mm were packed in a plastic bag (200 x 270 mm) and stored at 5 °C for 12 days. Therefore 4 different storage conditions were applied: under light and 500 mbar residual air (light + oxygen), under light and vacuum (20 mbar residual air) (light + vacuum), in the dark (wrapped in aluminum foil) and 500 mbar residual air (dark+ oxygen), in dark and vacuum (dark + vacuum).

II.3 Color measurement

Color was investigated at the beginning of storage and after 2, 4, 8 and 12 days. The color measuring system Chroma Meter CR-200 (Konica Minolta, Japan) was used. The system measured the color through three light impulses (L-, a*, b*) whereby the a*-value was the most important for determining the reddish color. Every slice was measured at the core in triplet. For the statistical analyses means and standard deviations were calculated (n=9).

II.4 Microbiological analysis

Microbial growth behavior was analyzed over a storage period of 5 weeks. Therefore, samples were taken from the core of the ham slices. Approximately 10 g sample/slice were weighted in a stomacher bag (VWR International, Germany), diluted 1:10 gravimetrical in peptone water (Carl Roth, Germany) and homogenized by using the stomacher for 90 s (IUL instruments, Spain). The homogenized samples were plated on Plate Count Agar (AppliChem, Germany) (48 h, 37 °C) and on Baird Parker Agar (Carl Roth, Germany) (72 h, 37°C) which was used for the staphylococcal count by using an automated spiral plater (Don Whitley Scientific Limited, UK). The colony forming units (cfu) were counted by using the aCOLyte colony counting device (Synbiosis, UK).

II.4 Nitrite and Nitrate Analysis

The analysis were carried out according to Schoch et al. [5] with slight modifications in the sample preparation. 15 g samples were cut in small cubes and weighted into glasses. Afterwards 10 mL borax solution (disodium tetraborate, Merck, Darmstadt, Germany) was added and stored at 5 °C until the analysis were carried out.

III. RESULTS AND DISCUSSION

Figure 1 shows the staphylococcal counts during processing and storage of the hams. At the beginning of processing, the hams with injected starter cultures reached staphylococcal counts of approx. 10^5 cfu/g meat, whereas the control hams without starter cultures (batch 1, 2) showed 2 log lower bacterial counts. At the end of processing, the staphylococcal counts resulted in approx. 10^2 cfu/g meat for batches without starter cultures (batch 1, 2) with no significant differences. Hams

with starter cultures (batch 3, 4) remained constant at a staphylococcal count of around 4 log cfu/g meat after 5 weeks of storage.

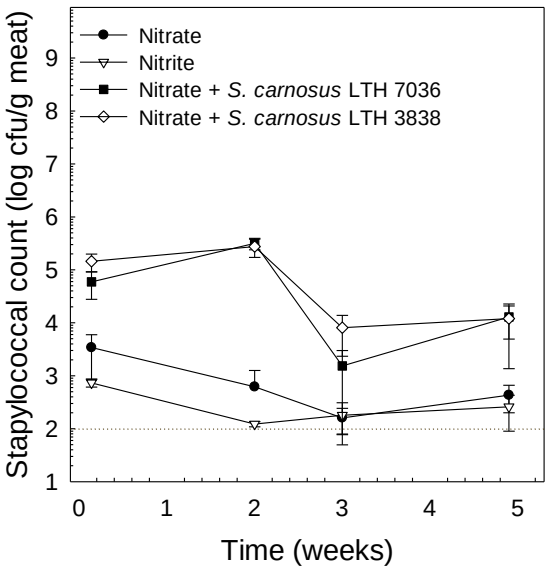

Figure 1: Staphylococcal counts investigated as cfu/g meat over time (week 0, 2, 3, 5) for each batch (n=4). Dotted line represent the limit of detection of 2 log cfu/g.

The presence of starter cultures or intrinsic microflora influences the trend of nitrate and nitrite during processing and storage of the raw hams. Figure 2 illustrates the nitrate content of the cured raw hams over time. Till day 14 the concentrations of nitrate increased for all samples cured with nitrate (batch 1, 3, 4). For the raw hams cured with nitrite, the nitrate content increased to approx. 50 mg/kg which can be explained by the oxygenation of some nitrite parts to nitrate (3). During drying, resting, and ripening the different nitrate reductase activities of the bacterial strains had an effect on the nitrate content. Hams inoculated with *S. carnosus* LTH 7036, with the highest nitrate reductase activity showed the highest decrease in nitrate concentration to a level of about 50 mg/kg after 3 weeks. In contrast the hams with *S. carnosus* LTH 3838 (low nitrate reductase activity) showed a lower nitrate content of approx. 150 mg/kg after storage. The nitrate control (batch 1) without starter cultures showed the highest amount of nitrate after 14 days and a decrease in nitrate content after 20 days due to the growth of the intrinsic microflora. The raw hams only cured with nitrite remained on a low nitrate level.

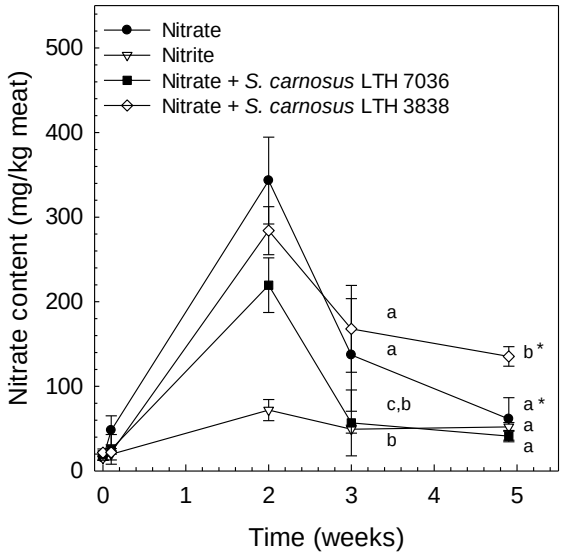

Figure 2: Nitrate content (mg/kg meat) over time for all batches (n=12). Letters represent significant differences within the batches at one point of time and * show differences within one batch during the last seven days.

Furthermore, pictures (Figure 3) and color measurements (Figure 4, 5) showed that the hams cured with LTH 7036 (high nitrate reductase activity) had significant higher a*-values than the other samples. This effect was observed for vacuum storage under bright and dark conditions.

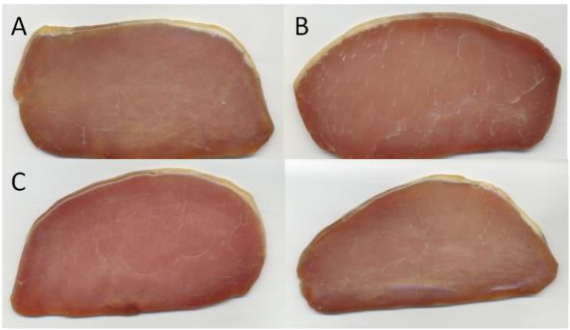

Figure 3: Ham slices after 12 days of storage at 5 °C, stored in vacuum package under light. A: Nitrate; B: Nitrite; C: Nitrate + *S. carnosus* LTH 7036; D: Nitrate + *S. carnosus* LTH 3838

The color stability of hams produced with nitrate and LTH 7036 was the highest during the 12 days of storage at 5 °C. This can be explained by

the reduction of nitrate during processing. Therefore, a higher content of nitrite is present in these hams and reacts with the meat pigment myoglobin to nitrosylmyoglobin leading to the typical red color. The same behavior was also observed in previous (results not shown) studies in which hams where stored under residual oxygen.

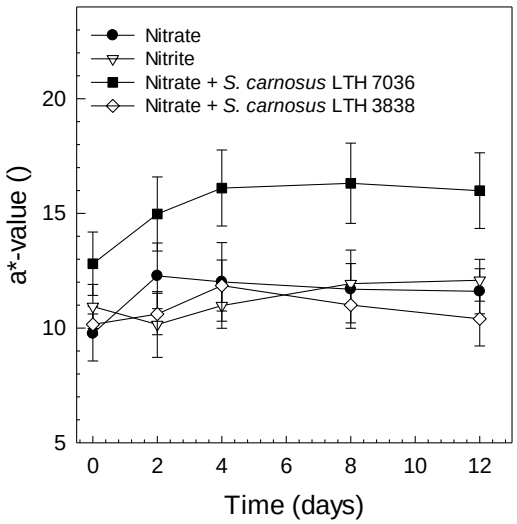

Figure 4: a*-values of ham slices determined over 12 days of storage at 5 °C. Stored in vacuum packages with light.

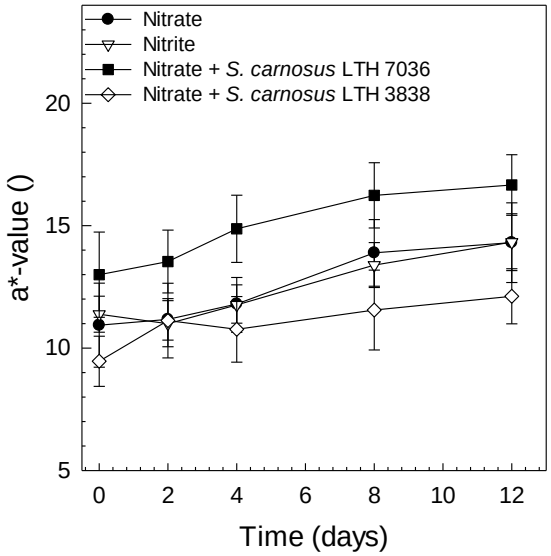

Figure 5: a*-values of ham slices determined over 12 days of storage at 5 °C. Stored in vacuum packages in the dark.

IV. CONCLUSION

The results showed that the application of starter cultures in combination with nitrate in raw cured hams by multi needle injection is possible. Moreover, the nitrate content during processing is highly influenced by the presence of microorganisms due to their nitrate reductase activity. Cured ham slices produced with high nitrate reductase active starter cultures (LTH 7036) formed a better color and lead to a higher color stability under various storage conditions compared to the hams without nitrate active starter cultures (LTH 3838). This leads to a better appearance and thus higher acceptance by the consumer for pre-packed raw cured hams.

ACKNOWLEDGEMENTS

This research project was supported by the German Ministry of Economics and Energy (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn). Project number AiF 17687 N.

REFERENCES

1. Pegg, R.B., *CURING Production Procedures*, in *Encyclopedia of Meat Sciences*, W.K. Jensen, Editor 2004, Elsevier: Oxford. p. 349-360.
2. Shahidi, F. and A.G.P. Samaranayaka, *CURING Brine*, in *Encyclopedia of Meat Sciences*, W.K. Jensen, Editor 2004, Elsevier: Oxford. p. 366-374.
3. Hammes, W.P., *Metabolism of nitrate in fermented meats: The characteristic feature of a specific group of fermented foods.* Food Microbiology, 2012. **29**(2): p. 151-156.
4. Müller, A., A. Weiß, and H. Schmidt, *Personal Communication: Physiological properties of Staphylococcus carnosus strains isolated out of fermented meat and fish products*, 2014: University of Hohenheim, Department of Microbiology and Hygiene. Stuttgart.
5. Schoch, A., M. Gibis, and A. Fischer, *Entwicklung einer HPLC/UV Methode zur Bestimmung von Nitrit und Nitrat in Fleischerzeugnissen und Vergleich mit der enzymatischen Methode.* Lebensmittelchemie, 2000. 54(2): p. 31.