POTENTIAL OF *STAPHYLOCOCCUS CARNOSUS* TO CONTROL MICROBIOLOGICALLY INDUCED PORE FORMATION IN COOKED HAM: LIMITATIONS

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Abstract - Pore formation in cooked ham can be caused by gas forming bacteria. We hypothesized that beside protective cultures such as *Lactobacillus sakei* (SafePro-B2®; used in a former study) also starter cultures belonging to *Staphylococcus ssp.* may have the potential to control microbiologically induced pore formation. The injection of brine containing *Staphylococcus carnosus* to meat resulted in cooked ham which was characterized by a large number of pores (approx. 2.27 ± 0.422 pores/cm²; pore size approx. 0.53 ± 0.090 mm²). Thus, *S. carnosus* seems to be unsuitable to control microbiologically induced pore formation in cooked ham. For this reason, the impact of SafePro-B2® on pore formation observed in the presence of *S. carnosus* was determined by inoculating the brine with both microorganisms (10^7-10^8 CFU/mL) prior to meat injection. The results showed that pore formation in cooked ham possibly caused or boosted by the addition of *S. carnosus* could significantly be decreased by SafePro-B2®. The contamination of brine and equipment was excluded. However, as the gas test of *S. carnosus* was negative as expected, the mechanisms of pore formation observed in the presence of *S. carnosus* could not be entirely clarified.

Key Words - Cooked ham, Pore formation, *Staphylococcus ssp.*

- INTRODUCTION

The optical appearance of packaged cooked ham slices is almost the only thing on which the consumers' purchasing decision depends on, and is thus one of the major quality parameters of the final product. Therefore, pore formation in cooked ham is a major problem for the meat industry because consumers associate this with a low quality of the product. Potential reasons that are responsible for pore formation maybe of technological and/or microbiological (e.g. contamination of brine) origin, which are additionally affected by the properties of the raw material such as its pH value or water content [1].
Starter cultures are widely used in the food industry to improve the microbial safety and organoleptic properties of products [2]. In the raw sausage manufacturing process, the applied starter cultures generate acid (preservative and important for cut resistance) and flavor compounds [3], [4]. For instance, *Staphylococcus carnosus* has a positive impact on the reddening of raw sausages as well as on their aroma profile [2].

Lipolysis and the release of free fatty acids are partly linked to the presence of microbial enzymes. Free fatty acids are the main precursors of oxidation products and hence important for flavor development [5]. Moreover, it has been shown that the addition of *S. carnosus* to raw meat can significantly decrease the maturation time of raw sausages [5].

Currently, the use of *Lactobacillus sakei* and *S. carnosus* in cooked ham is not very common but preliminary studies show promising effects regarding the shelf life of the final products when applying these bioprotective starter cultures [6].
Due to the ability of *S. carnosus* to reduce nitrate to nitrite, this bioprotective starter culture can also be used to produce meat products without conventional nitrite pickling salt [7].

We hypothesize that microbiologically induced pore formation in cooked ham can potentially be controlled by the bioprotective starter culture *S. carnosus*.

- MATERIALS AND METHODS

Production of cooked ham: Ham was produced from boneless trimmed pork loin muscle, which was injected with brine (± microorganisms). The brine consisted of approx. 83% water, 11.5% nitrite curing salt and 5.6% Schinken Top LP (Gewuerzmueller, Germany) containing sucrose, di-/triphosphate, sodium ascorbate, monosodium glutamate and flavor. The injected ham was tumbled at 2°C under 80% vacuum (~ 200 mbar) for 3 h and kept overnight in a tumbler (Vakona-Hermsen GmbH, Germany) under the same conditions. After 1 h storage at room temperature to simulate longer production times in the industry, a delta-T heat treatment was performed in a heating chamber (Ness Unigar 1800 BE, Germany) until a core temperature of 70°C was reached. Thus, the chamber temperature was at least 10°C higher than the internal temperature, but did not exceed 74°C. Afterwards the ham was cooled down in a refrigeration room at 2°C for 24 h.

Microorganisms: The bioprotective starter culture SafePro-B2® (*Lactobacillus sakei*) and the starter culture Bactoferm CS 300® (*Staphylococcus carnosus*) were provided by Christian Hansen (Denmark).

Inoculation of brine: The brine was inoculated with either *S. carnosus* (10^7-10^8 CFU/mL) or with a combination of *S. carnosus* (10^7-10^8 CFU/mL) and SafePro-B2® (10^7 CFU/mL).

Microbiological sampling and analysis: Samples were analyzed from fresh meat (raw material), brine, meat after brine injection, meat after tumbling, meat before heating and at different internal temperatures during the delta-T-heat treatment (at 20, 30, 40, 50, 60, and 70°C). For sample collection, three approx. 1 cm thick slices were cut from the ham after each processing step. The first slice was removed to exclusively count bacteria from the center of the ham. Therefore, a sample (approx. 10 g) was stamped out of the center of the respective slice using a round template with a diameter of 3.7 cm. Samples were then put into sterile stomacher bags, diluted with 90 mL sterile peptone water and tumbled for 2 min at 300 rpm in a stomacher. To determine bacterial counts of SafePro-B2®, appropriate dilutions were plated on MRS agar using a spiral plater (Don Whitley, UK) and anaerobically incubated at 30°C for 48 h. Samples were also plated on Chapman agar to determine count numbers of *S. carnosus* after aerobic incubation at 37°C for 24 h. Moreover, samples from fresh meat were plated on plate count agar and incubated aerobically at 30°C for 48 h. If a low bacteria count was expected, the plates were additionally spread plated due to a detection limit of the method at 10^2 CFU/mL. Counting of colonies was performed using an automatic counter (Acolyte, Synbiosis, Germany).

Gas test: A simplified gas test was performed using Durham tubes to detect microbial gas formation in MRS and Standard-1 nutrient broth. The broth was inoculated either with a colony of SafePro-B2® or *S. carnosus* and incubated at 30°C or 37°C for 48-72 h, respectively. The test was regarded positive when gas formation was detected in the Durham tubes.

Evaluation of pores in cooked ham: For the analysis of pore formation, each cooked ham was cut into 2 mm thick slices (n = 28 slices / ham) and scanned (Epson v100 Perfection, USA). The area of each slice was calculated by using ImageJ and pores with a size of 0.1 – 5.0 mm² were exclusively counted in the area of a defined inner rectangle of the ham slice. Based on this, the porosity, which was defined as the number of pores per area of ham slice, could be calculated.

Sensory evaluation: 26 panelists evaluated both the porosity (visual impression) of all manufactured cooked hams and the taste (triangle test) of products manufactured with the starter culture SafePro-B2® or *S. carnosus* in comparison to a control ham which was produced without

addition of microorganisms. Results were statistically evaluated over a one-way single factor analysis of variance (ANOVA).

- RESULTS AND DISCUSSION

The microbiological quality of the raw meat used in this study was found to be very good and only bacterial counts of $\leq 10^3$ CFU/g were determined. The starter culture *S. carnosus* was added to the brine prior to injection at concentrations of approx. 10^7- 10^8 CFU/mL which is a recommended inoculation level for meat [6]. Regarding the porosity, significant differences were found between hams produced without (control) or with the addition of *S. carnosus* as illustrated in Figure 1.

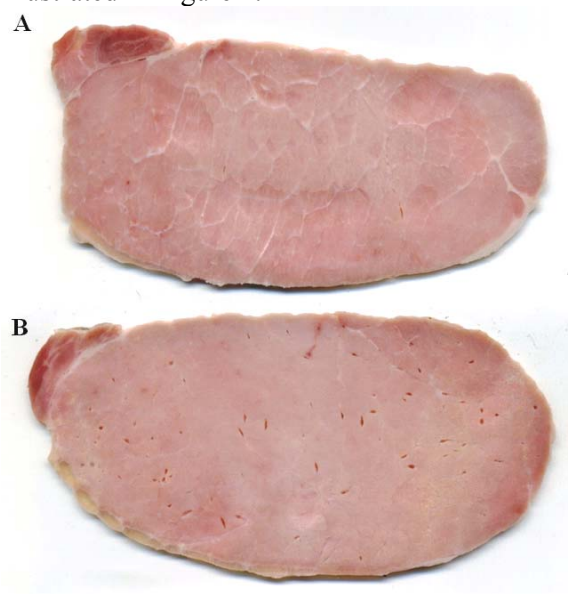

Figure 1 A: Ham initially injected with brine containing no starter cultures (control).
B: Ham produced with brine inoculated with 10^8 CFU/mL of the starter culture *S. carnosus* (Bactoferm CS 300®).

Overall, more than 2 pores /cm² were found in hams produced with *S. carnosus* (**Table 1**) with pore sizes of approx. 0.53 ± 0.09 mm².

Table 1 Porosity values of cooked hams produced with or without addition of the bioprotective cultures *L. sakei* (SafePro-B2®) and/or *S. carnosus* (Bactoferm CS 300®).

Cooked Ham Samples (± microorganisms)	Mean Porosity (pores/cm²)	SD
Control (no microorganisms)	0.28	0.12
10^6-10^7 CFU/g SafePro-B2®	0.29	0.11
10^7 CFU/g *S. carnosus*	2.27	0.42
10^6 SafePro-B2® + 10^7 CFU/g *S. carnosus*	0.93	0.42

However, we could not entirely explain this high porosity value. As commonly known and also confirmed by our gas test, *S. carnosus* is not able to form gas, neither in broth nor in the meat matrix. To exclude a potential contamination of the equipment, control hams without

microorganisms were always produced in between hams to which *S. carnosus* was added. These control hams were always characterized by a very low porosity of 0.28 ± 0.12 pores/cm². Thus, we can rule out that the observed pore formation was not due to microbial equipment contamination.
We also examined the impact of the bioprotective starter culture SafePro-B2® on the pore formation possibly caused or likelier boosted by *S. carnosus* in cooked hams. As demonstrated in Table 1, the porosity values between hams produced without microorganisms (control) or with SafePro-B2® were more or less equal. Furthermore, the porosity values of cooked hams produced with *S. carnosus* decreased significantly when *S. carnosus* and SafePro-B2® were simultaneously injected into raw meat prior to the tumbling process (Figure 2).

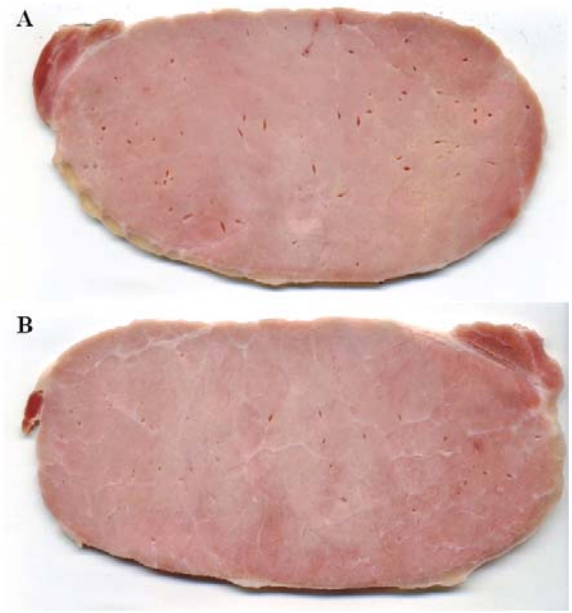

Figure 2 A: Ham produced with brine inoculated with 10^8 CFU/mL of the starter culture *S. carnosus* (Bactoferm CS 300®).
B: Ham produced with brine inoculated with both 10^8 CFU/mL of the starter culture *S. carnosus* and 10^7 CFU/mL of *L. sakei* (SafePro-B2®).

However, standard deviations (SD) gained from different hams produced with the combination of both cultures differed considerably. It should therefore be noted, that pore formation could indeed be significantly decreased but yet not reliably controlled by SafePro-B2® in the presence of *S. carnosus.* Regarding the determined bacterial counts it should also be mentioned that *S. carnosus* ($7.73*10^5$ KBE/g at 60°C) was less sensitive to high temperatures then the culture SafePro-B2® ($< 1*10^2$ KBE/g at 60°C).

The statistical evaluation of porosity and taste can be summarized as follows: Panelists found significant differences between the control hams and hams produced with *S. carnosus* in terms of porosity. In contrast to that, no differences were detected between the control and hams produced with SafePro-B2®. However, the simultaneous injection of *S. carnosus* led again to products with a significantly higher porosity.

Regarding the evaluation of taste, no off flavors were detected in either hams produced without addition of starter cultures or in cooked hams which were initially injected with brine containing SafePro-B2® or *S. carnosus* (Bactoferm CS 300®).

- CONCLUSION

S. carnosus seems by the current standpoint not to improve pore formation in cooked ham. More research is needed to clarify the mechanisms of pore formation. In contrast, the bioprotective culture SafePro-B2 (*L. sakei*) seems to be a promising culture to control microbiologically induced pore formation in cooked ham.

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