CONTROL OF MICROBIOLOGICALLY INDUCED (*Lactobacillus brevis*) PORE FORMATION IN COOKED HAM USING STARTER CULTURES (*Lactobacillus sakei*)

M. Loeffler[1*], N. Rahn[1], M. Gibis[1], K. Herrmann[1], H. Schmidt[2] and J. Weiss[1]

[1] Department of Food Physics and Meat Science, Institute of Food Science and Biotechnology, University of Hohenheim, Stuttgart, Germany

[2]Department of Food Microbiology, Institute of Food Science and Biotechnology, University of Hohenheim, Stuttgart, Germany

Abstract - Pore formation in cooked ham may be attributed to microbiological or technological reasons and leads to low quality final products. We hypothesized that microbiological induced pore formation could be controlled by addition of protective starter cultures, more precisely *Lactobacillus sakei* (SafePro-B2®). The use of SafePro-B2® in cooked ham led to a product which was comparable in appearance to control ham slices without pores. However, the addition of a typical gas forming bacteria (*Lactobacillus brevis*) led to a significant pore formation in cooked ham, both at high concentrations of 10^5 CFU/g meat and at concentrations of 10^2 CFU/g meat simulating an initial contamination of brine. The porosity was significantly decreased when SafePro-B2® was additionally added to the brine and injected into meat prior to the tumbling process. This also increased the consumer´s purchasing decision. Moreover, the sensory evaluation of cooked hams revealed that there were no significant differences between the hams produced with and without addition of starter cultures. Consequently, the use of SafePro-B2® in cooked ham has an outstanding potential to control microbiological induced pore formation and its application might be of great interest to the meat industry.

Key Words - Pore formation, Cooked ham, Protective starter cultures

I. INTRODUCTION

Cooked ham is one of the most popular meat products in Europe and America but it is also gaining popularity in China [1]. The optical appearance of packaged cooked ham is almost the only thing on which the consumers' purchasing decision depends on. Therefore, pore formation in cooked ham is a serious problem for the meat industry because consumers associate this with a low quality of the product. The mechanisms of pore formation in cooked ham are yet not fully understood. Potential reasons could be both technological and microbiological (e.g. contamination of brine) aspects. Furthermore, pore formation may be affected by the properties of the raw material such as its pH value or water content [2].

Commercial starter cultures are "generally recognized as safe" (GRAS), which states that these microorganisms are safe under the conditions of the intended use [3]. The application of starter cultures improves the microbial safety of meat products due to growth limitations of food pathogens. Depending on the used microorganisms, starter cultures offer also important advantages regarding e.g. organoleptic product properties [4]. Currently, the use of starter cultures in cooked ham is not very common. However, it has been shown that the application of *Lactobacillus sakei* and *Staphylococcus carnosus* to porcine muscle prior to the heat treatment of hams improves the shelf life of the final products [5].

Moreover, the processed meat can retain its natural and organic status upon addition of starter cultures. Natural or organic meat is produced without the addition of nitrite, which normally inhibits growth of food borne pathogens. The addition of natural nitrate and *S. carnosus* as a starter culture to ham has been shown to be as effective as the use of conventional nitrite pickling salt against the growth of *Clostridium perfringens* due to the ability of *S. carnosus* to reduce nitrate to nitrite [6].

We hypothesize that pore formation in cooked ham may be caused by gas forming bacteria even at low bacterial counts, and that microbiologically induced pore formation can be controlled by the addition of starter cultures such as *L. sakei*.

II. 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% (~ 200 mbar) vacuum for 3 h and kept overnight in a tumbler (Vakona-Hermsen GmbH, Germany) under the same conditions. After 1 h storage at room temperature, a delta-T heat treatment was performed in a heating chamber (Ness Unigar 1800 BE, Germany) according to **Figure 1** until a core temperature of 70°C was reached. Afterwards the ham was cooled down in a refrigeration room at 2°C for 24 h.

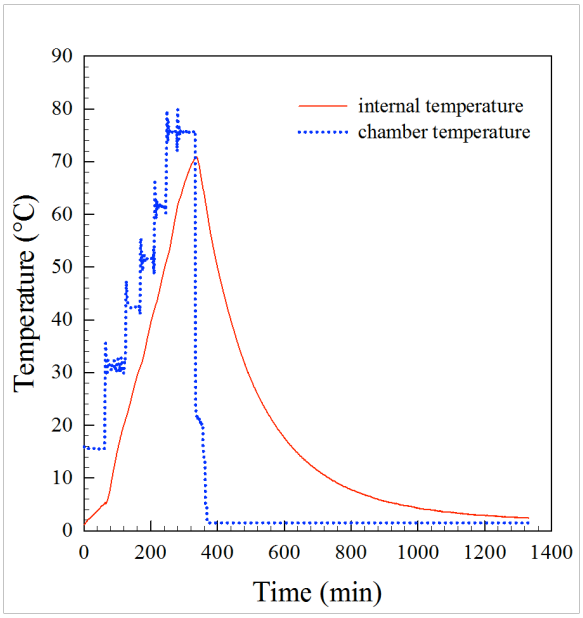

Figure 1. Delta-T heat treatment (in steam) during ham production process. 1 h prior to the heating process, samples were left at room temperature to simulate longer production times in the industry.

Microorganisms: The bioprotective starter culture SafePro-B2® (*Lactobacillus sakei*) and the gas forming bacteria Cocktail 055 (gas forming strain *Lactobacillus brevis)* were provided by Christian Hansen (Denmark).

Inoculation of brine: The brine was inoculated with either SafePro-B2® (10^7 CFU/mL) or *L. brevis* (10^3 or 10^6 CFU/mL). Moreover, combinations between SafePro-B2® and *L. brevis* were applied together considering mentioned inoculation levels.

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. Appropriate dilutions were plated on MRS agar using a spiral plater (Don Whitley, UK) and anaerobically incubated at 30°C for 48 h. Samples from raw 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).

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) and the taste (triangle test) of hams manufactured with the starter culture SafePro-B2® in comparison to a control ham which was produced without

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

III. RESULTS AND DISCUSSION

Prior to the ham manufacturing process, the quality of the used raw meat was examined by determining the initial bacterial cell counts which were constantly ≤ 10^3 CFU/g. This adhered with the EU regulations for pork meat. The starter culture SafePro-B2® containing the strain *L. sakei* was added to the brine prior to injection at concentrations of approx. 10^7- 10^8 CFU/mL, which is a commonly found and recommended inoculation level for meat [5]. In samples taken from hams after an internal temperature of 60°C was reached during the delta-T heat treatment, less than $2.4*10^4$ CFU/g of *Lactobacillus sakei* could be detected. After reaching 70°C, all microorganisms were inactivated. Regarding the porosity values, no significant differences were found between hams produced without (control) or with the addition of the starter culture SafePro-B2® as shown in Table 1.

Table 1 Porosity values of cooked hams produced with or without addition of a protective starter culture (SafePro-B2®) and/or a gas forming bacteria strain (*L. brevis*).

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
10^2 CFU/g *L. brevis*	1.06	0.29
10^5 CFU/g *L. brevis*	1.00	0.44
10^6 SafePro-B2® +10^2 CFU/g *L. brevis*	0.35	0.11
10^6 SafePro-B2® +10^5 CFU/g *L. brevis*	0.97	0.41

To induce microbial pore formation in cooked ham, the meat was inoculated with brine containing either 10^3 CFU/mL or 10^6 CFU/mL (equal to 10^2 or 10^5 CFU/g meat, respectively) *L. brevis* a heterofermentative, lactic acid bacteria, which is able to produce CO_2. The first concentration was chosen to simulate a contamination of brine. Long storage times of brine at around 7°C prior to injection into meat may support microbial growth to levels high enough to cause pore formation in cooked ham as was shown by Comi et al. [7]. In their study, *Weisella viridescens* was found to produce gas even at low concentrations like the one used in this study. The second inoculation level was chosen to simulate the effect of pore formation if very high concentrations of gas forming bacteria would be present in the brine. Up to an internal temperature of 50°C, bacterial counts remained at approx. 10^2 CFU/g ($1.86*10^2$ CFU/g) when *L. brevis* was initially used at concentrations of 10^2 CFU/g meat, while for the higher inoculation level cell counts remained at approx. 10^5 CFU/g ($1.30*10^5$ CFU/g). At 60°C, almost all bacteria were already inactivated. As demonstrated in **Table 1**, the porosity values of hams produced with *L. brevis* significantly increased in comparison to the ones determined for the control and for hams produced with SafePro-B2®. In contrast, no significant differences were determined between hams produced with different inoculation concentrations of *L. brevis*. However, comparison of the pore sizes showed that the higher inoculation concentration of *L. brevis* increased the pore size (Table 2).

Table 2 Determined pore sizes of cooked hams produced with and without addition of the gas forming strain *L. brevis* and in combination with SafePro-B2®.

Cooked Ham Samples (± microorganisms)	Mean Pore Size (mm²)	SD
10^2 CFU/g *L. brevis*	0.47	0.10
10^5 CFU/g *L. brevis*	0.53	0.12
10^6 SafePro-B2® +10^2 CFU/g *L. brevis*	0.42	0.14
10^6 SafePro-B2® +10^5 CFU/g *L. brevis*	0.48	0.12

As already mentioned, *L. brevis* can produce CO_2 which expands with increasing temperature. During the heat treatment of hams, meat proteins denature. Thus, the formed gas gets entrapped in the meat matrix and is hence visible as pores in the final product. To avoid microbiological induced pore formation by *L. brevis*, SafePro-B2® was additionally added to the brine to potentially control growth of the gas forming bacteria and thus to decrease the number and size of pores in cooked hams. Figure 2A presents a cooked ham which was initially injected with brine containing 10^3

CFU/mL of *L. brevis*, whereas 2B presents the final product when the meat was injected with brine containing a mixture of *L. brevis* (10^3 CFU/mL) and SafePro-B2® (10^7 CFU/mL).

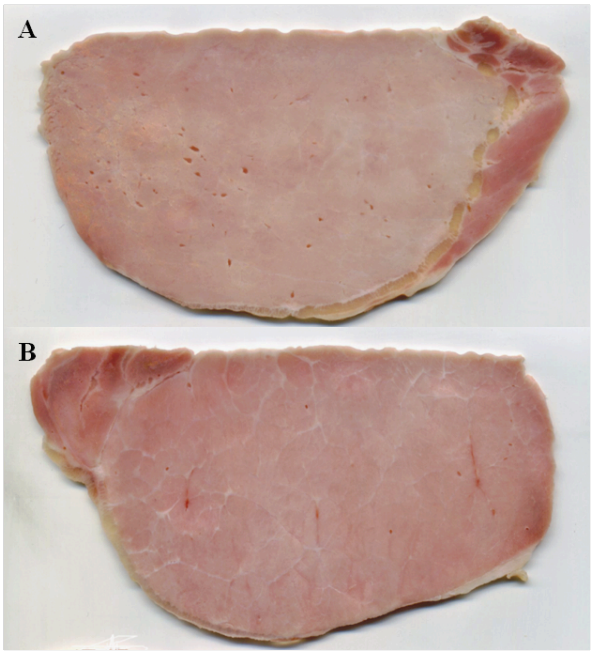

Figure 2. **A**: Ham initially injected with brine containing 10^3 CFU/mL of *L. brevis,* simulating a contamination of brine, and **B:** Ham produced with brine which was inoculated with 10^3 CFU/mL of *L. brevis* and 10^7 CFU/mL of the starter culture SafePro-B2®.

The starter culture SafePro-B2® (*L. sakei*) can significantly decrease porosity and pore size of cooked hams (Figure 2), which is also in accordance with results from Table 1 and 2. Moreover, microbial induced pore formation as a consequence of brine contamination was successfully controlled.

Three results from the sensory evaluation should be highlighted. First of all, panelists did not find any significant differences between the control hams and hams produced with SafePro-B2® in terms of porosity. However, ham produced with the CO_2 forming culture *L. brevis* in combination with SafePro-B2® showed a statistically significant difference compared to hams without protective cultures. This was shown to positively influence the consumer´s purchasing decision. While only 20% of the surveyed participants would have bought ham to which initially 10^2 CFU/g *L. brevis* was added, this number was increased to 88% when SafePro-B2® was additionally added. Furthermore, off flavors were not detected in both hams produced without addition of starter cultures and cooked hams which were initially injected with brine containing SafePro-B2®.

IV. CONCLUSION

Gas forming bacteria such as *L. brevis* can contaminate brine and form gas even at low concentrations of 10^2 CFU/g meat. During ham production, the formed gas gets entrapped in the matrix and final products are hence characterized by a high porosity. Starter cultures such as SafePro-B2® (*L. sakei*) can be used to successfully control pore formation induced by *L. brevis*, without changing the characteristic taste of cooked ham.

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