IMPACT OF TWO *STAPHYLOCOCCUS CARNOSUS* STRAINS IN CURED RAW HAM ON KEY AROMA COMPOUNDS, COLOR, AND SENSORY QUALITY

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Abstract – The volatile profile of North European cured raw hams inoculated with two *Staphylococcus carnosus* strains with different physiological characteristics was studied: one with a high proteolytic activity and one with a high nitrate reductase. As hypothesis, the combination of these strains may show positive effects on color and aroma development. Following analyses were conducted: microbial analyses (total viable counts, staphylococcal counts), volatile key compounds by using Headspace-Trap Gas Chromatography, color measurements of L*, a*, and b* values, and a sensory test. The positive effect of both *S. carnosus* cultures resulted in an increase of the concentrations of 3-methylbutanal, benzaldehyde, and acetophenone. The higher levels of aroma compounds in cured raw hams with a mixture of both *S. carnosus* strains were confirmed by significant higher scores in the sensory test. In addition, the levels of the lipid oxidation products nonanone and nonanal was limited by the inoculation of the strain with high nitrate reductase activity and the combination of both *S. carnosus* strains.

Key Words – Aroma compounds, nitrate reductase activity, cured raw ham

I. INTRODUCTION

The curing process of cured North European raw hams is traditionally performed by dry- or wet-curing or by combining dry- and wet curing with sodium chloride and sodium nitrite and/or potassium nitrate [1]. While the application of starter cultures in dry-cured fermented sausages is a standard process [2], the usage in cured raw ham is yet not often applied. However, an acidification of cured raw hams due to the application of lactic acid bacteria is undesired. The bacterial group of coagulase-negative staphylococci is more suitable for application in a whole muscle system, such as cured raw ham, due to their nitrate reductase activity to speed up color generation and stabilize the color pigments in cured meat products [3]. Additionally, the development of volatile aroma compounds of fermented sausages was strain-specific and the content of aroma compounds increased by adding staphylococci as starter cultures [4]. We hypothesized that a combination of a proteolytic strain and a strain with high nitrate reductase activity may increase amino acid degradation products, improve color, and limit the formation of lipid oxidation compounds.

MATERIALS AND METHODS

Selection of starter cultures: S. carnosus LTH 3838, a *S. carnosus* subsp. *utilis* isolate of fermented fish with the ability to digest sarcoplasmatic proteins [5], and *S. carnosus* subsp. *carnosus* LTH 7036 from fermented sausages were chosen because of the high nitrate reductase activity (0.72 mol NO_2^- per $1x10^7$ cfu) [5]. Cell suspensions with approximately 1×10^{11} cfu/mL of both strains were prepared by centrifugation.

Cured raw ham production: Fresh pork loins (*M. longissimus dorsi*, pH: 5.55 ± 0.11 were purchased from a local wholesaler market (MEGA, Stuttgart, Germany). 4 batches of cured raw hams were manufactured by either injecting brine without starter cultures as control, with *S. carnosus* LTH 3838, with *S. carnosus* LTH 7036 (approx. 10^7 cfu/mL brine) or with a mixture of both *S. carnosus* strains (starter mix, ratio 1:1, each ~5×10^6 cfu/mL). The brine of each batch was prepared with 10% (w/w) nitrite curing salt (Südsalz, Heilbronn, Germany; 0.9% $NaNO_2$) and was added to reach an inoculum of 1×10^6 cfu/g meat. For the indented injection weight of 10% (w/w), all hams were injected using a multi-needle injector type 105 MC2 R (105 needles, 2 mm diameter, 0.7 bar injection pressure; Günther, Dieburg, Germany). Subsequently, a dry-curing step was applied to obtain a total curing salt content of 40 g curing salt per kg meat. After

injection of the brine, hams were subjected to curing for 1 week at 5 °C. A first drying step (7 days; 10 °C – 15 °C; 75 – 85% relative humidity) and smoking (at 24 °C) was conducted in a climatic chamber Air Master UK-1800BE (Reich, Urbach, Germany). Then, hams were dried (15 °C, 75% rel. humidity) to approximately 22% (w/w) weight loss after 3 weeks and vacuum stored for the ripening of additional 4 weeks at 15 °C. Duplicate experiments and analyses were carried out.

Microbial analysis: Samples were taken on day 0 (after brine injection), day 21 (start of ripening) and day 49 (end of ripening). The detection of colony forming units (cfu) was carried out on Plate Count agar for the total viable count (AppliChem, Darmstadt, Germany). Cultures were incubated at 37 °C for 48 h as well as on Baird Parker agar (Carl Roth, Karlsruhe, Germany) which is mixed with 10 mL of 1% potassium tellurite solution (Sigma Aldrich, Steinheim, Germany), and incubated at 37 °C for 72 h for the staphylococcal count.

Determination of key volatile compounds: A Headspace-Trap Gas Chromatography-Flame ionization detector system (Perkin Elmer, Rodgau, Germany) (TurboMatrix 40 Trap Headspace sampler directly coupled to Clarus® GC 580) was used for the detection of volatile aroma compounds (2-butanone, 3-methyl-butanal, 2-pentanone, acetoin, hexanal, 1-oecten-3-ol, benzaldehyde, nonanone, nonanal, and acetophenone) as described by Bosse *et al.* [6]. Aroma analysis was performed for each sample in triplicate, after 21 days (start of ripening) and 49 days (end of ripening).

Color measurement: CIE L*a*b*-values of the cured raw hams were performed by using a Chroma Meter CR 200 (Minolta, Osaka, Japan). Three slices of cured raw hams of every batch (from core) were measured 3 times.

Sensory analysis: The sensory attributes, overall aromatic odor and taste, were evaluated by 20 trained sensory panelists at the beginning (day 21) and the end of ripening (day 49). Therefore, the core samples (2 mm thick, diameter 25 mm) of the inoculated cured raw hams were presented at ambient temperature. Before evaluation, the control was savored as reference sample (set to score 5). The sensory analysis was conducted in duplicate by using a quantitative descriptive analysis with line scale from 0 (less) to 10 (high) intensive aroma.

Statistical analysis: The statistical tests (Kruskal-Wallis ANOVA - analysis of variance - on ranks) for color values were performed with Sigmaplot® 12.5 (Systat Software Inc., San Jose, CA, USA).

II. RESULTS AND DISCUSSION

The mean injection weights of all batches were 9.4 ± 1.7% with an average pH of the raw meat/pork loin of 5.55 ± 0.11 that led to similar starting conditions for all batches of cured raw hams. During the process, a mean weight loss of 21.5 ± 1.4% was attained. All inoculated cured raw hams were comparable in moisture content (mean 64.0 ± 1.4%) to the control.

In Fig. 1, the total viable and staphylococcal counts are shown. During processing, the bacterial counts of starter hams decreased about half log level for the total viable count and about 2 log levels for the staphylococcal counts at the start of ripening (day 21) compared to the count after injection.

In contrast, the intrinsic microbiota of the control ham grew until the end of ripening up to 5.3 log cfu/g and 3.7 log cfu/g for total viable and staphylococcal counts, respectively.

Figure 1. Total viable count (A) and staphylococcal count (B) in log cfu/g meat for all cured raw ham batches after injection (day 0), at beginning of ripening (day 21) and at end of ripening (day 49) (n = 8)

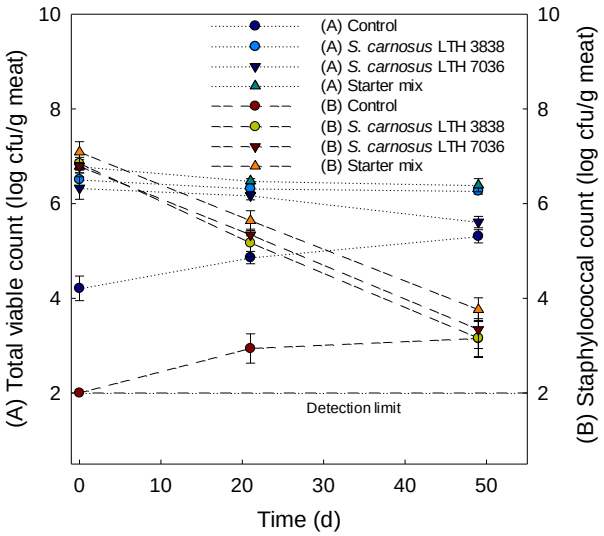


Table 1 shows the color values of the cured raw

hams. For *S. carnosus* LTH7036 and the starter mix, a significant increase in redness (a*value) was measured. This fact is a result of the high nitrate reductase activity of the strain LTH 7036. All batches showed a significantly time-dependent increase in the most L*, a*, and b*-values during ripening.

In Fig. 2, the analyzed 8 key aroma compounds are shown. During ripening, the amount of mushroom-like odorous 1-octen-3-ol alcohol significantly increased (8 ng/g meat) for the hams inoculated with the starter mix.

The aroma compounds 3-methylbutanal and benzaldehyde are typical aldehydes, which are formed as Strecker degradation products of the amino acids leucine or isoleucine, and phenylalanine, respectively [7]. Intrinsic enzymes of meat and bacterial peptidases can accelerate the release of these amino acids. The content of benzaldehyde in cured raw hams inoculated with the mixture of both strains was significantly higher (50 ng/g) than in all other hams studied at the start and end of ripening. Moreover, the benzaldehyde level of the cured raw hams inoculated with the proteolytic strain (LTH 3838) increased. Therefore, the type of starter culture significantly affected the content of benzaldehyde.

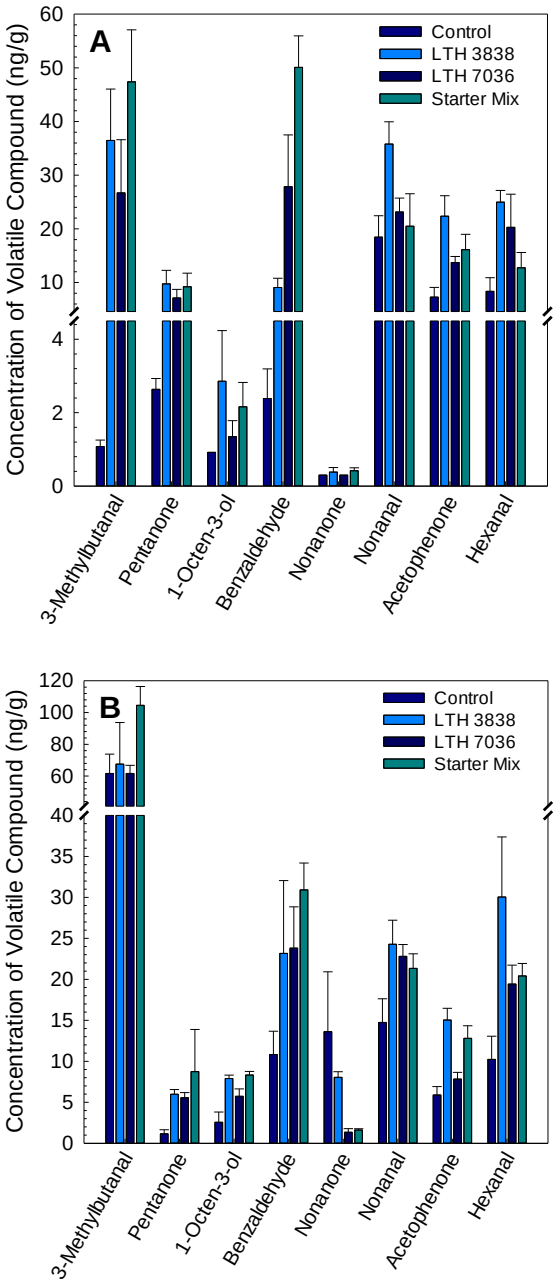
Table 1 Color measurements of L*a*b* values of the control, *S. carnosus* LTH 3838, *S. carnosus* LTH 7036, and the starter mix (LTH 3838 + LTH 7036) at the beginning (21 days) and end of ripening (49 days)

Batch	Time (d)	L*[1] ()	a*[1] ()	b*[1] ()
Control	21	45.0 ±1.21^{ad}	10.9 ±0.85^{a}	6.3 ±0.64^{a}
	49	49.1 ±0.78^{bc}	13.5 ±0.83^{bd}	7.9 ±0.59^{b}
S. carnosus LTH 3838	21	46.4 ±1.65^{ad}	10.4 ±0.88^{a}	6.5 ±1.07^{ad}
	49	50.8 ±2.14^{c}	13.1 ±0.84^{b}	9.4 ±1.13^{c}
S. carnosus LTH 7036	21	44.1 ±3.00^{a}	12.6 ±1.44^{b}	6.4 ± 0.86^{a}
	49	47.2 ±3.17^{bd}	15.8 ±2.03^{c}	8.7 ±0.93^{bc}
Starter mix	21	45.3 ±1.30^{ad}	12.9 ±1.01^{b}	6.2 ±0.58^{a}
	49	49.0 ±1.12^{b}	15.6 ±1.01^{cd}	7.8 ±1.01^{bd}

[1] different letters indicate significant differences ($p<0.05$)

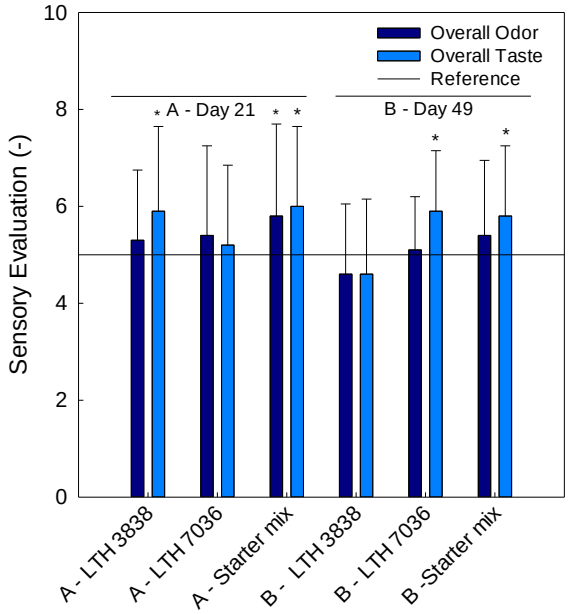
In cured raw hams inoculated with starter cultures, higher levels of 3-methylbutanal were detected than in control hams and the highest content was found in hams inoculated with the starter mix (day 21). At the end of ripening, highest content of 3-methylbutanal was determined for the starter mix ham (Fig. 2B). Both levels of benzaldehyde and 3-methylbutanal were significantly higher when both strains were inoculated together. This fact verifies our hypothesis.

Figure 2. Concentration of the key aroma compounds of cured raw ham batches without cultures (control), with *S. carnosus* LTH 3838, *S. carnosus* LTH 7036, or with both strains (starter mix) at day 21 (A) and day 49 (B) of ripening



The hams inoculated with the proteolytic strain showed highest hexanal content and the mixture of both strains lowest hexanal content. At the end of ripening, highest levels of hexanal and nonanal as typical fatty acid oxidation products [4] were detected in cured hams inoculated with *S. carnosus* LTH 3838, whereas lowest contents were detected in control hams. Nevertheless, from all inoculated hams, the hams inoculated with the starter mix and with the strain LTH 7036 showed lowest levels of nonanal and hexanal. This fact may result in the high activities of the enzymes nitrate reductase and catalase, which can contribute to a limitation of lipid oxidation [4]. The same trend was observed for 2-pentanone and acetophenone. During ripening, the 2-butanone and nonanone content of all cured raw hams significantly increased (Fig. 2B). The level of acetophenone was highest for the cured raw ham inoculated with *S. carnosus* LTH 3838 and the mixture of both *S. carnosus* strains due to the higher proteolytic activity of strain LTH 3838. The positive effect of the combination of both strains was shown by an increase in levels of 3-methylbutanal, acetophenone, 1-octen-3-ol, benzaldehyde, and 2-pentanone due to higher proteolytic enzymatic activities that was caused by the strain LTH 3838. Excepting the control, a limited lipid oxidation was observed via nonanone, hexanal, and nonanal content due to higher nitrate reductase and catalase activity added because of the strain LTH 7036.

Figure 3. Sensory evaluation for the overall odor and taste of all cured raw ham batches;* indicates significant differences between reference score 5 after 21 days (A) and 49 days (B)



In Fig. 3, the results of the sensory evaluation are shown. The proteolytic strain LTH 3838 improved the sensory quality at beginning and the strain LTH 7036 increased the sensory attributes at the end of ripening. Consequently, the starter mix ham showed significantly highest sensory acceptance for overall taste at the beginning and at the end of ripening.

III. CONCLUSION

The inoculation of a starter mix with high proteolytic activity and high nitrate reductase activity already can improve the color and aroma profiles at the initial and final step of ripening. The results indicate that it is possible to reduce the ripening time of cured raw hams with intensive flavor.

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