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Influence of homopolysaccharide-producing lactic acid bacteria on the spreadability of raw fermented sausages (onion mettwurst)

Jonas Hilbig, Lisa Hildebrandt, Kurt Herrmann, Jochen Weiss, and Myriam Loeffler

Abstract: The purpose of the study was to investigate the effect of a reduced pH value (5.1 instead of 5.5 to 5.6) on the properties of highly perishable, spreadable raw fermented sausages (onion mettwurst) with or without the addition of homopolysaccharide (HoPS)-producing lactic acid bacteria (LAB). Hence, sausages with HoPS-producing LAB and a pH value of 5.1 were produced and compared to sausages (pH 5.1) produced with a non-exopolysaccharide (EPS)-forming strain (*Lactobacillus sakei* TMW 1.2037). Microbial growth and pH values were monitored during processing (24 °C for 48 hr, 10 °C for 24 hr) and storage (14 days at 0 to 2 °C). Furthermore, fat (Weibull–Stoldt) and EPS contents were determined in the final products. Sausages were characterized using texture profile and sensory analysis. The fat contents ranged from 16% to 19% and the determined EPS concentrations ranged from 0.17 to 0.59 g/kg for *L. sakei* TMW 1.411 and *Lactobacillus curvatus* TMW 1.1928 and from 0.67 to 1.58 g/kg for *L. curvatus* TMW 1.51. The strains *L. sakei* TMW 1.411 and *L. curvatus* TMW 1.51 reduced the hardness of the samples significantly ($P < 0.05$) compared to the control samples. Regarding spreadability and mouthfeel, sausages containing an EPS-forming culture were rated slightly better than the control samples and the taste was not negatively influenced.

Keywords: exopolysaccharides, *in situ* formation, lactic acid bacteria, spreadable raw fermented sausage

Practical Application: This study clearly demonstrated that it is promising to apply HoPS-producing LAB to maintain the spreadability of pH-reduced (pH 5.1) spreadable raw fermented onion mettwurst, which may prospectively give the opportunity to increase the safety of this highly perishable product.

1. INTRODUCTION

Raw fermented sausages are made from minced meat, which is mixed with spices and additives before filling into casings. The type of raw fermented sausages produced in this study was the traditional German "Zwiebelmettwurst," henceforth referred to as onion mettwurst (Feiner, 2006; Leistner, 1995). In this product, onion, fresh or powdered, is added for the typical taste of the product (Feiner, 2006). The pH value of this kind of spreadable raw fermented sausage is not or only slightly decreased during manufacturing (approximately pH 5.5 to 5.6) to maintain the spreadability of the product (Prändl, Fischer, Schmidhofer, & Sinell, 1988). Due to the slight decrease in pH during production and the lack of sufficient other hurdles, this sausage is a highly perishable product and, because of that, a decrease in the pH value could result in an increased safety of the product. At the pH value of 5.5 to 5.6, the proteins are charged negatively, producing a product with a good spreadability (Hamm, 1996). However, at lower pH values, the isoelectric point of the meat proteins is reached (the average isoelectric point of meat proteins is 5.0) and cross-links are formed (Ruusunen & Puolanne, 2005). Due to this effect and the low fat content of the sausage (approximately 15%), the spreadability may be lost. Hilbig, Gisder, et al. (2019) demonstrated in a recently performed study that the application of *in situ* exopolysaccharide (EPS)-producing lactic acid bacteria (LAB) can significantly increase the spreadability of fat-reduced spreadable raw fermented sausages (teewurst). Furthermore, the application of EPS-producing LAB was found to weaken the structure of reconstructed ham (Hilbig, Loeffler, Herrmann, & Weiss, 2019b). Due to these promising results, EPS-producing LAB may also have the potential to maintain the spreadability of onion mettwurst with a lower pH (5.1). EPS are polysaccharides synthesized by bacteria, including LAB having GRAS status (Czaczyk & Myszka, 2007; Rühmkorf, Rübsam, et al., 2012). EPS can be distinguished into two different groups: the homopolysaccharides (HoPS) and the heteropolysaccharides (HePS). HePS are synthesized out of different substrates in an energy-intensive and complex biosynthesis, which is related to the cell wall biosynthesis (Sutherland, 2001). Since Hilbig et al. (2019b) concluded that HoPS-producing LAB showed the best results to maintain the spreadability of fat-reduced spreadable raw fermented sausages, only HoPS-producing LAB were used in the present investigation. HoPS are synthesized by glucosyltransferases out of sucrose and, furthermore, they are composed of only one monomer type D-glucose or L-fructose (2012 Rühmkorf, Jungkunz, Wagner, & Vogel, 2012; Rühmkorf, Rübsam, et al., 2012). Compared to HePS, the production of HoPS is less energy intensive and complex, and higher concentrations can therefore be synthesized (Korakli, Pavlovic, Gänzle, & Vogel, 2003). Due to the *in situ* production of the EPS during fermentation of the sausage, they do not have to be labeled as an additive, thus meeting the demand of the consumer for natural and healthy food without additives (Rownan, 2016). Because the

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sausages are produced from raw meat with a high pH value (5.5 to 5.6) and other missing hurdles like preservatives or a low a_w-value, the risk of contamination and growth of pathogenic microorganisms and due to this production of toxins are high (Leistner, 1978). Furthermore, the meat offers ideal growth conditions because it contains a high amount of nutrients and has a high water activity, because these kind of sausages are not, or only slightly, dried (Sofos, 2008; Tompkin, 2002). In microbiological analyses done by Friedrich, Sabrowski, Layer, and Lohneis (2012) and Kuleaşan and Çakmakçı (2002) in 2012, onion mettwurst was declared a high-risk product due to multiple instances of growth and survival of *Salmonella spp*., *Listeria monocytogenes*, and enterohemorrhagic *Escherichia coli* or verotoxigenic *Escherichia coli*. These pathogens all would lead to severe food poisoning that can be life threatening, especially for young, old, pregnant, and immunocompromised people. Some strains of LAB are able to produce bacteriocin, which could additionally inhibit the growth of pathogens (Bredholt, Nesbakken, & Holck, 2001; Casaburi, Di Martino, Ferranti, Picariello, & Villani, 2016; Schillinger, Kaya, & Lücke, 1991).

The aim of this study was to decrease the pH value of the raw fermented sausages and maintain the spreadability of the product through *in situ* EPS production by the applied LAB. Therefore, three different EPS-producing *Lactobacillus* strains (*Lactobacillus sakei* TMW 1.411, *Lactobacillus curvatus* TMW 1.1928, and *L. curvatus* TMW 1.51) were selected and compared to a non-EPS-producing strain (*Lactobacillus sakei* TMW 1.2037) as a control.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals. Peptone was purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Plate Count Agar (PCA; agar 15.0 g/L, glucose 1.0 g/L, peptones 5.0 g/L, and yeast extract 2.5 g/L) was obtained from AppliChem GmbH (Darmstadt, Germany). MRS agar, MRS broth (peptone from casein 10.0 g/L, meat extract 10.0 g/L, yeast extract 4.0 g/L; D(+)-glucose 20.0 g/L, dipotassium hydrogen phosphate 2.0 g/L, Tween® 80 1.0 g/L, diammonium hydrogen citrate 2.0 g/L, sodium acetate 5.0 g/L, magnesium sulfate 0.2 g/L, and manganese sulfate 0.04 g/L, for MRS agar additionally agar–agar 14.0 g/L), and Anaerocult® were purchased from Merck KGaA (Darmstadt, Germany). Dowex 50WX4 hydrogen form and Dowex 66 free base (ion exchange resins) were purchased from Sigma–Aldrich Chemie GmbH (Munich, Germany).

2.1.2 LAB strains. *Lactobacillus curvatus* TMW 1.1928, *L. curvatus* TMW 1.51, *L. sakei* TMW 1.411, and, as the non-EPS producing strain, *L. sakei* TMW 1.2037 (hereafter referred to as *L. curvatus* 1.51, *L. curvatus* 1.1928, *L. sakei* 1.411, and *L. sakei* 1.2037, respectively) were obtained from the University of Freising, Munich, Department of Technical Microbiology (TMW). All strains were maintained at –80 °C in 20 wt% glycerol. The strains used and the type of EPS that they produce are shown in Table 1.

2.1.3 Meat and spices. Pork meat was obtained from a local food retail market (MEGA eG, Stuttgart, Germany) and standardized to S III according to the GEHA meat classification system (Prändl et al., 1988). The spices mild paprika, white pepper, nutmeg, ginger, onion powder, and the additives nitrite curing salt (NCS, 0.5% nitrite), and the additives ascorbic acid, monosodium glutamate, and sucrose were purchased from Gewürzmüller GmbH (Ditzingen, Germany).

Table 1–Strains and incubation conditions prior to spreadable raw fermented sausage inoculation.

Strains	Type EPS	Incubation conditions
Lactobacillus curvatus TMW 1.1928 (cryoculture)	HoPS	MRS-bouillon 24 hr at 30 °C
Lactobacillus curvatus TMW 1.51 (cryoculture)	HoPS	
Lactobacillus sakei TMW 1.411 (cryoculture)	HoPS	
Lactobacillus sakei TMW 1.2037 (cryoculture)	No EPS-formation (control)	

2.2 Methods

2.2.1 LAB preparation. The *Lactobacillus* strains were activated in a 40 mL MRS broth for 24 hr at 30 °C. After the incubation, the solutions were centrifuged (Z32HK, HERMLE Labortechnik GmbH, Wehingen, Germany) at 5,000 rpm for 10 min and 20 °C. Afterward, the MRS broth was exchanged with 30 mL peptone water prior to use for the sausage production because a sensory evaluation was to be conducted, and the taste should not have been influenced.

2.2.2 Raw fermented sausage production. For each batch, 6 kg of pork meat (SIII), 26 g/kg nitrite curing salt, 4 g/kg white pepper, 0.5 g/kg glutamate, 0.3 g/kg nutmeg, 0.5 g/kg ginger, 1 g/kg paprika mild, 0.5 g/kg ascorbic acid, and 4 g/kg onion powder were used. The target pH value was set to be 5.1 and, because of that, different amounts of sucrose were needed for the different strains to achieve it: for the control strain *L. sakei*, 1.2037 0.25 g/kg sucrose, for *L. sakei* 1.411 and *L. curvatus*, 1.1928 1 g/kg, and for *L. curvatus*, 1.51 5 g/kg. The sugar contents were determined in a prescreening (data not shown). The chilled meat (SIII) was minced with a 3-mm hole plate in a meat grinder (Type W-114, Maschinenfabrik Seydelmann KG, Stuttgart, Germany). After mincing, the meat was mixed with the spices, additives, and LAB (approximately 10^8 CFU/mL) in a precooled meat mixer (Kneader mixer RC-40, Mainca, Barcelona, Spain) for 5 min (with alternating directions every 30 s for the first 3 min). Afterward, the sausage batter was filled (Stuffer: MWF 591, MADO Patron, Dornhan, Germany) into polyamide casings with a caliber of 43 (Budenheimer Kunststofftechnik GmbH & Co. KG, Schweighofen, Germany). The sausages were fermented for 48 hr at 24 °C and for 24 hr at 10 °C in a smoke chamber (Unigar 1800 BE, Ness & Co. GmbH, Remshalden, Germany). After the production, the sausages were stored at 0 to 2 °C in a cold storage room until examination. The experiments were repeated using the same formulation and the same experimental conditions; however, different raw materials (different lots of meat, bacteria, and spices) were used. In Figure 1, the process of the sausage production is shown.

2.2.3 Determination of the fat content. To determine the fat content, the Soxhlet fat extraction method according to Weibull–Stoldt was applied (BfR, 2004). The meat sample was crushed in a KitchenAid and 10 g was always mixed with 150 mL of 4 N HCL. The mixture was cooked at 115 °C for 1 hr. Afterward, each sample was washed with hot distilled water through a double layer of fluted filters until the pH of the filtrate was neutral. The filters, which then contained the fat, were put into extraction shells and dried at 60 °C for at least 12 hr. The extraction shells were put into the Soxhlet extraction chamber and petroleum ether was filled into the extraction flasks. The samples were extracted

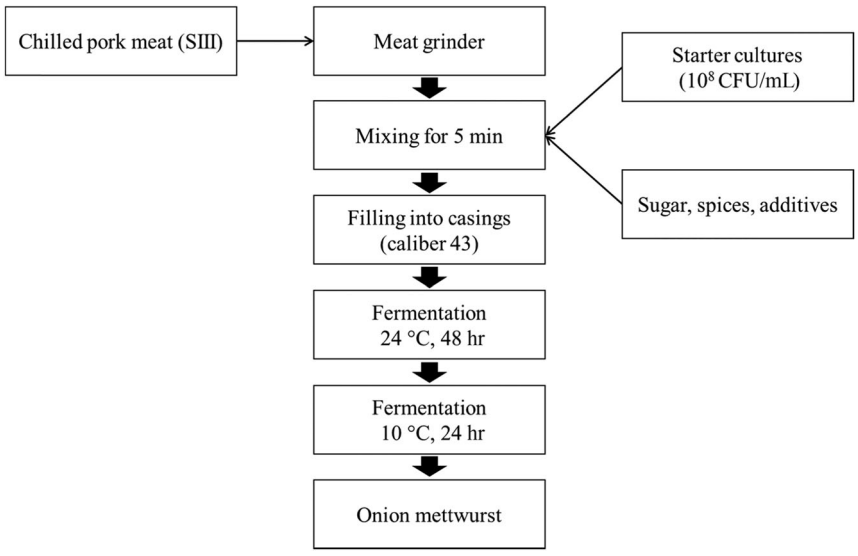

Figure 1–Process flow chart of the production of raw fermented sausages (onion mettwurst).

for 4 hr. The weight of the flasks was obtained before and after the extraction.

2.2.4 Microbiology. Aerobic cell counts were determined after the filling of the sausages, after fermentation (72 hr), and after 7 and 14 days of storage. Anaerobic cell counts were determined after the filling of the sausages, after 6, 24, 48, and 72 hr of fermentation and after 7 and 14 days of storage at 0 to 2 °C. For this purpose, 90 g of peptone was mixed with 10 g of the respective meat sample in a Stomacher bag and homogenized for 60 s with six strokes per second with a Stomacher (IUL Instrument GmbH, Königswinter, Germany). Dilutions of the samples were plated on MRSA plates and on PCA plates with an automated spiral plater (Don Whitley Scientific Limited, West Yorkshire, UK). The plates were either stored under anaerobic (MRSA) or aerobic (PCA) conditions for 24 to 48 hr at 30 °C. The colonies were automatically counted using Acolyte (Synbiosis, Cambridge, UK).

2.2.5 pH measurement. The pH value of the raw minced meat, during fermentation (after filling, 6, 24, 30, 48, and 72 hr) and after 7 and 14 days of storage, was determined using a penetration electrode and a pH meter (WTW GmbH, Weilheim, Germany).

2.2.6 Isolation and quantification of EPS. The amount of EPS present in the samples was determined according to the method provided in the publication of Hilbig et al. (2019b). The sausage samples were dried and crushed, and for each sample 10 g was dissolved in 20 mL of ethanol and incubated for 12 hr at 4 °C. Thereafter, the samples were centrifuged and the pellets then suspended in 5 mL of distilled water. First, the proteins in the samples were precipitated with 20% trichloroacetic acid, and then the supernatants were mixed with 2 volumes of ethanol for the precipitation of the polysaccharides (EPS). After centrifugation, the resulting pellets were dissolved in bidistilled water (ddH_2O) and perchloric acid (70%) to reach a final concentration of 5% of perchloric acid (v/v), and the EPS then hydrolyzed for 6 hr at 95 °C in a water bath. Afterward, the solutions were treated with a mixture of a weak anionic and a strong cationic ion exchanger (mixture 1:1) to remove salts and other ions from the solution. Finally, the solutions were analyzed by HPLC using a Rezex RHM column (Rezex RHM, Phenomenex, Aschaffenburg, Germany) with a flow rate of 0.6 mL/min (ddH_2O) at 75 °C and an injection volume of 20 µL, and detected with a refractive index detector at 40 °C. The HoPS results were compared to a glucose standard curve.

2.2.7 Texture profile analysis. For the texture profile analysis (TPA), slices of 1.5-cm thickness were cut off from the sausages and the casing was removed from the slices. Subsequently, a cylinder with a diameter of 2 cm were stamped out with a metal pipe. Ten samples were taken for each batch and they were compressed to 50% of their original thickness in a double compression cycle test with a probe of 2.5-cm diameter, at a cross-head speed of 50 mm/min (Instron Model 3365 Tensile Tester, Instron GmbH, Darmstadt, Germany; equipped with 5 kN load cell). Furthermore, the force–time deformation curves were obtained and the time between the two compression cycles was 20 s. The hardness of the sample is determined as the first peak of the compression. The cohesiveness (stability of the sausage) and springiness index (deformation during the compression) were calculated with Equations (1) and (2):

$$\text{Cohesiveness } (-) = \frac{\text{Second area } (J)}{\text{First area } (J)}, \quad (1)$$

$$\text{Springiness index } (-) = \frac{(a - b)}{(c - d)}. \quad (2)$$

The parameters in Equation (2) are defined as follows: a is the distance to the maximal second compression (mm), b the distance to the onset of the second compression (mm), c the distance to the maximal first compression (mm), and d the distance to the onset of the first compression (mm).

2.2.8 Sensory evaluation. The sensory evaluation of the sausages was performed with a panel of 20 untrained people. The sausages were cut into 1.5-cm thick slices with a diameter of about 4.3 cm. For the evaluation, a paired comparison test was conducted, with the non-EPS-containing control sample corresponding to a score of 5. The other EPS-containing samples had to be rated compared to the control sample regarding

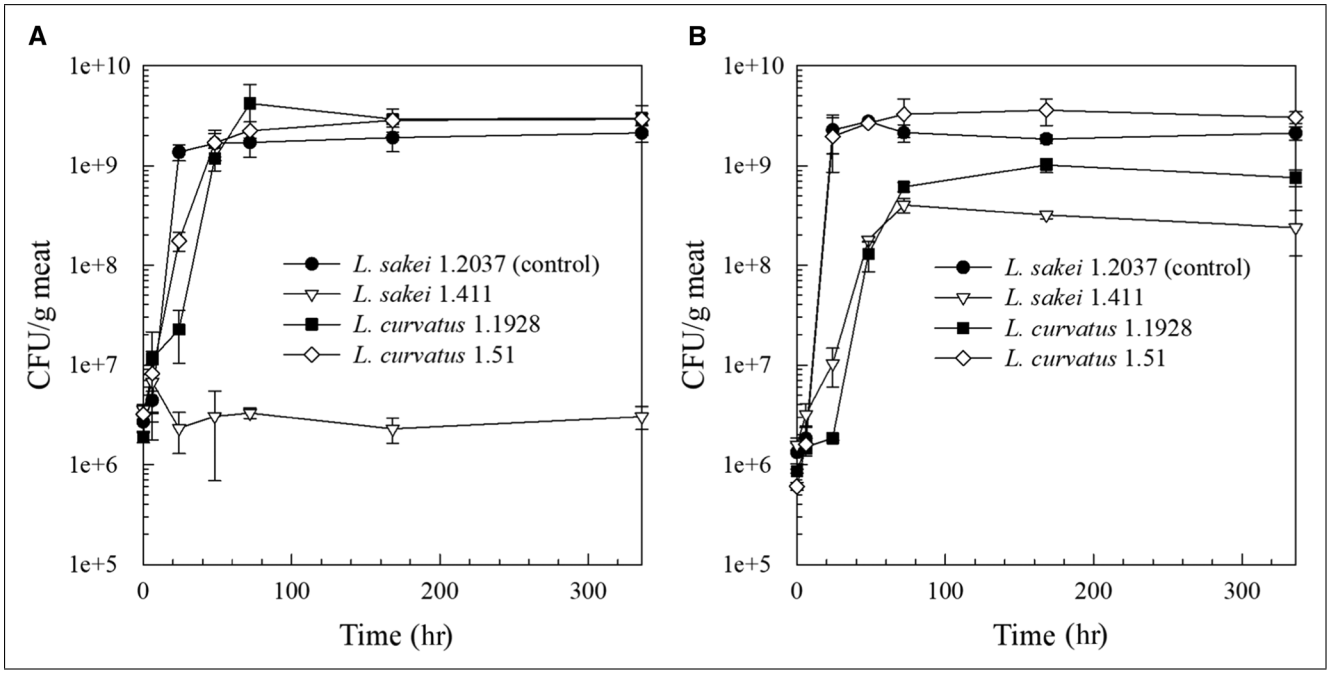

Figure 2–Anaerobic cell counts of the sausages produced with the HoPS-producing strains *Lactobacillus sakei* 1.411, *Lactobacillus curvatus* 1.1928, *L. curvatus* 1.51, and the non-EPS-producing strain *L. sakei* 1.2037 (control) during the fermentation (0 to 72 hr) and storage (72 to 336 hr) of the sausages of the first experiment (A) and the second experiment (independent sausage production) (B).

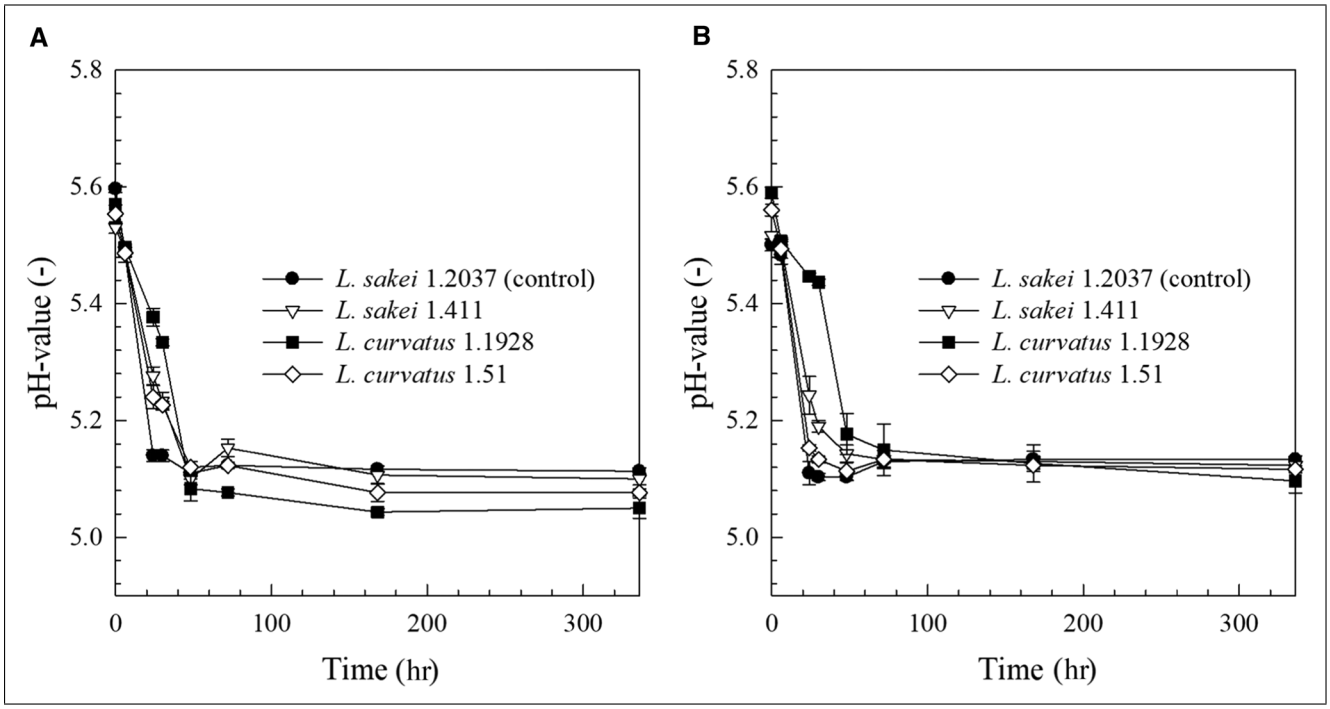

Figure 3–pH values of the sausages produced with the HoPS-producing strains *Lactobacillus sakei* 1.411, *Lactobacillus curvatus* 1.1928, *L. curvatus* 1.51, and the non-EPS-producing strain *L. sakei* 1.2037 (control) during the fermentation (0 to 72 hr) and storage (72 to 336 hr) of the sausages of the first experiment (A) and the second experiment (independent sausage production) (B).

hardness/spreadability, creaminess/mouthfeel, and taste, in a range from 0 (harder/less spreadable, disliked in creaminess, poorer in taste) to 10 (softer/more spreadable, favored creaminess, better taste). Two software, Fizz Acquisition 2.51 (Biosystems, France) and Fizz Calculations 2.50 (Biosystems, France), were used in the sensory evaluation.

2.3 Statistical analysis

All measurements were repeated at least two times using duplicate samples. Means and standard deviations were calculated from these measurements using Excel (Microsoft, Redmond, VA, USA). SPSS (IMB SPSS Statistics 24, IBM, Munich, Germany) software was used to statistically evaluate the results. A one-way

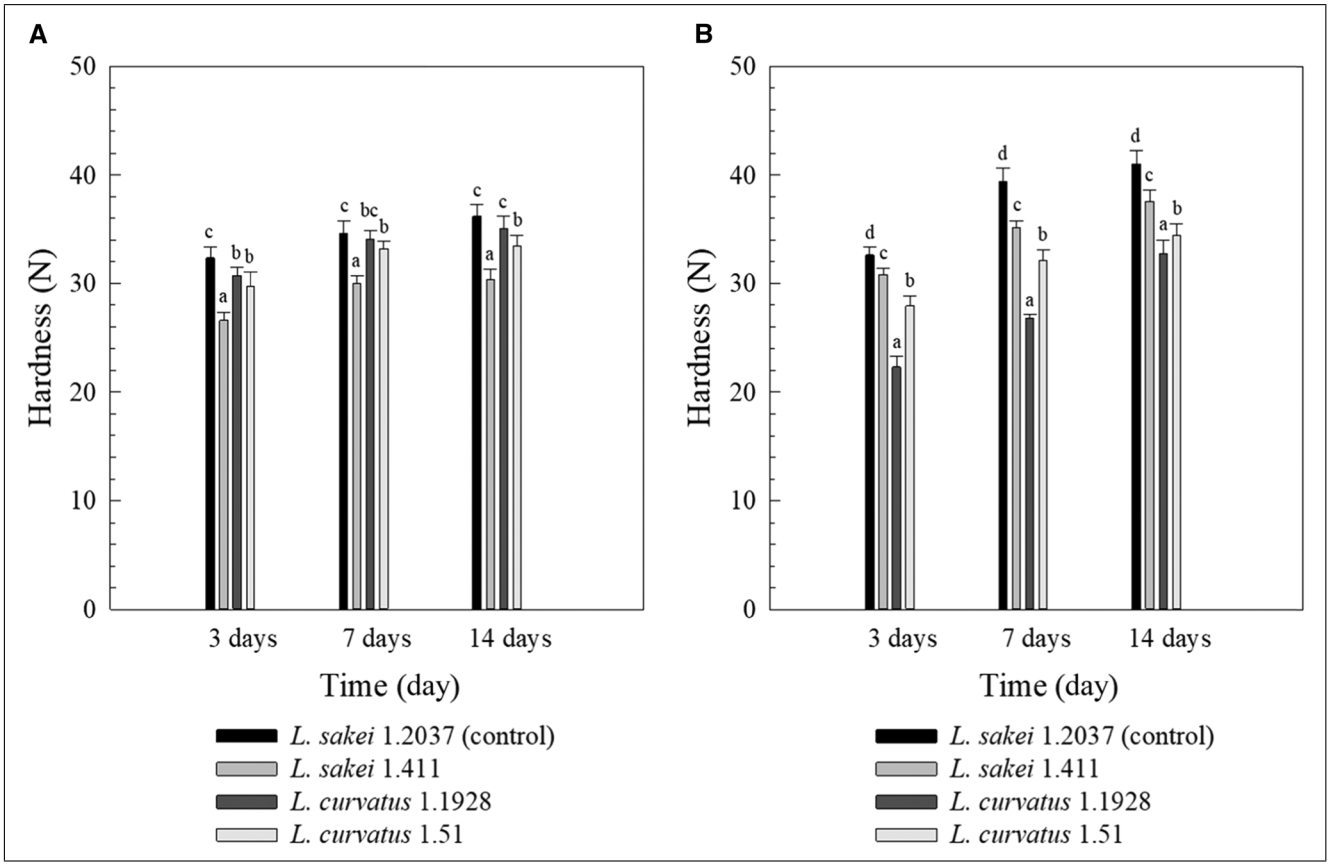

Figure 4–Hardness of the sausages produced with the HoPS-producing strains *Lactobacillus sakei* 1.411, *Lactobacillus curvatus* 1.1928, *L. curvatus* 1.51, and the non-EPS-producing strain *L. sakei* 1.2037 (control) of the first experiment (A) and the second experiment (independent sausage production) (B). Different letters indicate significant differences ($P < 0.05$).

Table 2–Quantification of the EPS content (HPLC) of sausages produced with *L. sakei* 1.411, *L. curvatus* 1.1928, and *L. curvatus* 1.51 after 48 and 72 hr of fermentation for the first (A) experiment and the second (B) experiment (independent sausage production). Polysaccharide content of the respective control samples has been subtracted.

		EPS content (g/kg dry matter)[a]	
Strain	Time	A	B
L. sakei 1.411	48 h	0.443 ± 0.010^{d}	0.375 ± 0.006^{c}
	72 h	0.252 ± 0.005^{b}	0.168 ± 0.005^{a}
L. curvatus 1.1928	48 h	0.360 ± 0.011^{c}	0.582 ± 0.016^{e}
	72 h	0.178 ± 0.005^{a}	0.257 ± 0.008^{b}
L. curvatus 1.51	48 h	1.533 ± 0.011^{h}	1.576 ± 0.006^{i}
	72 h	0.864 ± 0.005^{g}	0.660 ± 0.005^{f}

Note. Values with different letters show significant differences ($P < 0.05$) in between all samples.
[a]Numbers are means ± standard deviation from duplicates, each examined two times ($n = 4$).

ANOVA with a post hoc Tukey's test was performed for the quantification of the EPS, sensory evaluation, and the TPA to investigate significant differences between the samples and the control ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Microbiology and pH values

The anaerobic counts of the different LAB during fermentation (0 to 72 hr) and storage (72 to 336 hr) are shown in Figure 2. The cell counts increased for every strain from 10^6 to 10^9 CFU/g of meat within the first 48 hr and remained at that level. The cell counts of *L. sakei* 1.411, however, remained at 10^6 CFU/g meat during the whole experiment (Figure 2A). Although *L. sakei* 1.411 did not grow in the first experiment, higher cell counts (increase to 10^8 CFU/g meat) could be determined in the replicate (Figure 2B). The aerobic cell counts of the meat used were, for both experiments, around 10^4 CFU/g meat, indicating a good meat quality (Feiner, 2006). Erkkilä et al. (2001) investigated the influence of different LAB as the predominant fermentation organisms in dry fermented sausages. According to their results, the cell counts also increased from 10^6–10^7 to 10^8–10^9 CFU/g of meat during the fermentation (7 days; 19 to 23 °C) and ripening (28 days; 17 °C) time and the cell counts remained at the same level for 35 days. LAB need fermentable saccharides to proliferate, which was why sucrose was used in this experiment. The sugars are fermented to organic acids such as lactic acid (Leroy & De Vuyst, 2004; Stiles & Holzapfel, 1997). Figure 3 shows that the presence and growth of LAB reduced the pH value of the products. The pH value of the batter directly after the production was 5.5 to 5.6 and the desired final pH value of 5.1 was reached by all of the strains investigated (the sugar content needed was examined in a prescreening experiment; data not shown) after 48 hr of fermentation.

3.2 EPS quantification by HPLC

The quantification of the EPS showed differences between the different sampling times. For all strains, the EPS content was higher

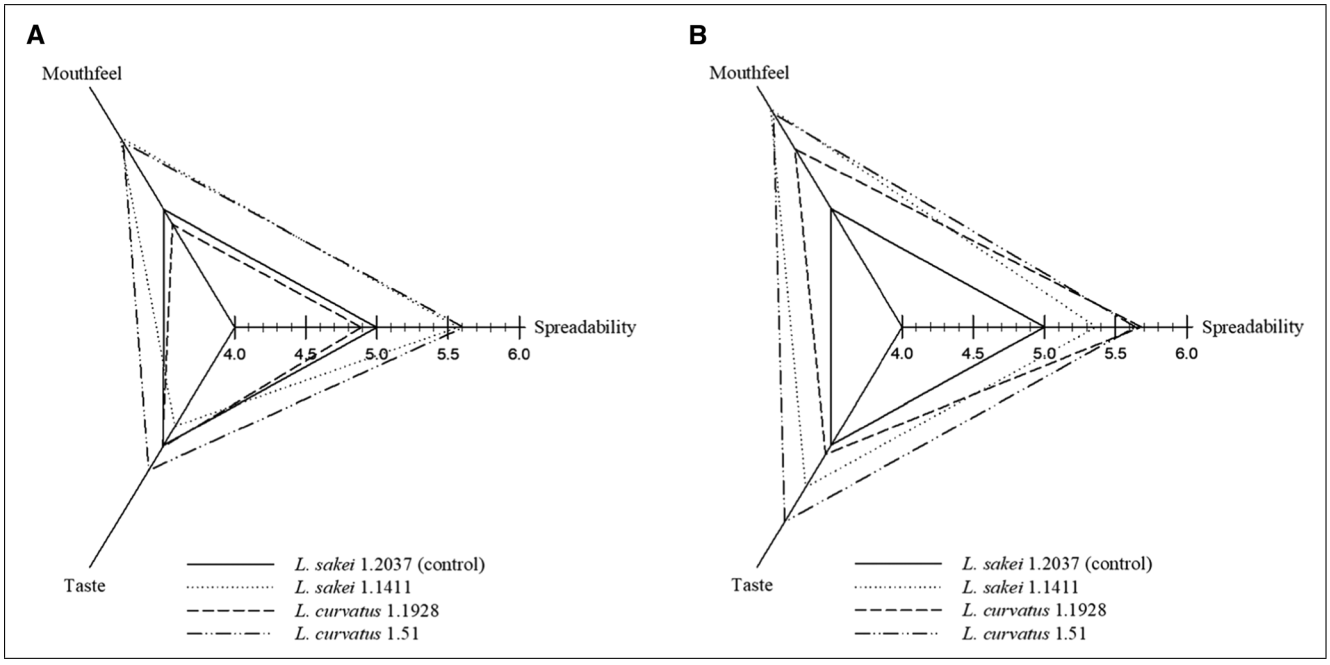

Figure 5—Sensory evaluation of the sausages produced with the HoPS-producing strains *Lactobacillus sakei* 1.411, *Lactobacillus curvatus* 1.1928, and *L. curvatus* 1.51 compared with their respective control samples (produced with the non-EPS-producing strain *L. sakei* 1.2037) of the first experiment (A) and the second experiment (independent sausage production) (B).

after 48 hr compared to 72 hr of fermentation. This could be also seen in the replicate of the experiment (Table 2). For example, the EPS content of the samples produced with *L. sakei* 1.411 was between 0.375 and 0.443 g/kg after 48 hr and 0.168 and 0.252 g/kg after 72 hr of fermentation. Hydrolytic or eliminase enzymes can break down the EPS polymers, which could explain the decreased contents after 72 hr of fermentation. Pham, Dupont, Roy, Lapointe, and Cerning (2000) showed that prolonged fermentation also led to lower EPS yields produced by *Lactobacillus rhamnosus* R because of enzymatic degradation. Moreover, for some HoPS-producing LAB the two-step enzymatic reaction of glycosyltransferase is not only able to polymerize but also to hydrolyze the product into the substrate (Korakli & Vogel, 2006). *Lactobacillus curvatus* 1.51 produced significantly more EPS compared to the other EPS-forming strains ($P < 0.05$), which could be due to the higher amount of saccharose needed to achieve the pH value of 5.1. van Geel-Schutten, Flesch, Ten Brink, Smith, and Dijkhuizen (1998) investigated the effect of increased saccharose contents on the production of EPS and the study showed that an increasing amount of available saccharose increases the EPS content produced by *Lactobacillus reuteri* strain 121 and 180. Some HoPS-producing strains are able to produce up to 40 g/L EPS under optimal conditions (30 °C for 60 hr), which was, for instance, shown for *Lactobacillus sanfranciscensis* LTH2590 in a sucrose-MRS medium (Korakli et al., 2003). Furthermore, the molecular weight of the EPS produced by *L. sakei* 1.411 was investigated by Prechtl, Wefers, Jakob, and Vogel (2018). The molecular weight of the produced EPS was influenced by the fermentation conditions and the highest molecular weight was determined at 10 °C with 3.0×10^8 Da followed by 30 °C with 1.8×10^8 Da, respectively.

3.3 Fat content and TPA

The fat content of sausages is an important factor for the spreadability and sensory properties of the product (Lücke, 2015). The sausages in the first experiment had a fat content of 16.31 ± 0.15% and a slightly higher one in the replicate (18.73 ± 0.15%), which was, in both cases, slightly higher than in most products available on the market having a fat content of 15% (Wahl, 1995). In order to be accepted by the consumers, the product has to fulfill important sensory attributes, including an appealing texture, appearance, and flavor (Pons & Fiszman, 1996). Hence, the textural properties of the produced raw fermented sausages were determined to investigate if the lower pH values have an influence on the texture of the onion mettwurst compared to control products without an EPS-producing LAB (*L. sakei* 1.2037). For cohesiveness and springiness, the samples did not show significant differences compared to the control sample ($P > 0.05$; data not shown). The results of the hardness determined by TPA are illustrated in Figure 4. The texture was determined after the fermentation (72 hr) and after 168 hr (7 days) and 336 hr (14 days) of storage at 0 to 2 °C. In the first experiment (Figure 4A), the sausages produced with *L. sakei* 1.411 had the softest texture compared to the other strains. The hardness of the samples was 26.6 N after 72 hr and increased to 30.4 N after 336 hr of storage. The values were significantly lower ($P < 0.05$) compared to the control sample at all investigated time points, with values of 32.4 N after 72 hr and 36.2 N after 336 hr of storage, respectively. The sausages were even significantly softer ($P < 0.05$) than the other EPS-containing samples in the experiment. The sausages produced with *L. curvatus* 1.51 also showed significantly lower ($P < 0.05$) values than the control in both investigations, whereas, for the strain *L. curvatus* 1.1928, this was only true for the replicate (Figure 4B). Nevertheless, samples containing *L. curvatus* 1.1928 led to the softest samples. Compared to the quantified EPS amounts, the strain produced in the first experiment had less EPS than in the replicate after the 72 hr of fermentation (0.178 g/kg compared to 0.257 g/kg). The sausages produced with *L. curvatus* 1.51 showed, in both experiments, similar hardness values without a big deviation, which could be due to the higher amounts of sugar used to reach the desired pH value of 5.1 compared to the other strains leading

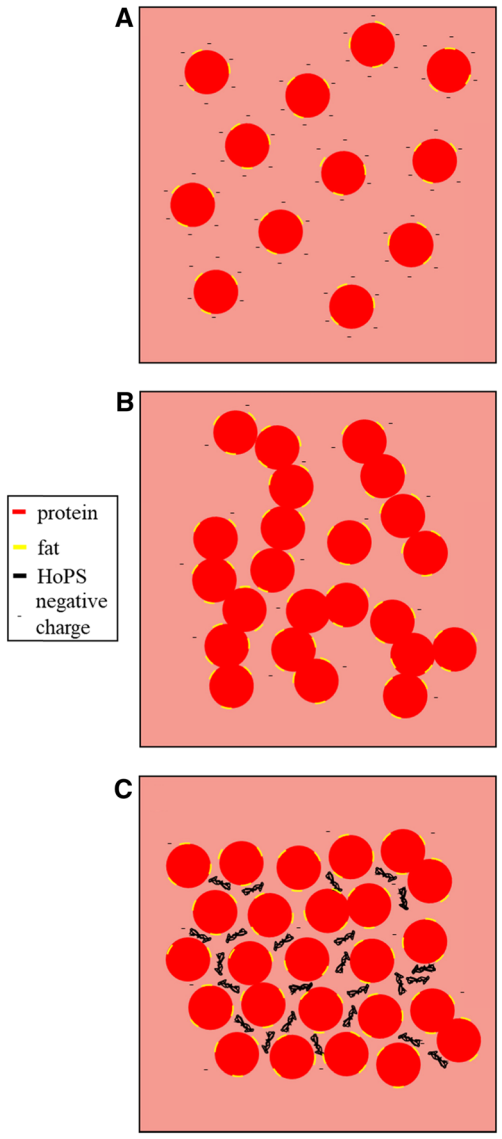

Figure 6–Proposed mechanism of the interactions among protein, fat, and EPS in the produced sausages either with the HoPS-producing strains *Lactobacillus sakei* 1.411, *Lactobacillus curvatus* 1.1928, *L. curvatus* 1.51, or the non-EPS-producing strain *L. sakei* 1.2037 (control) (red, protein; yellow, fat; black, EPS; -, charge).

to higher concentration of EPS (0.864 and 0.660 g/kg). This was also shown in the study done by van Geel-Schutten et al. (1998). Ahmed, El Soda, Hassan, and Frank (2005) investigated cheese produced either with or without EPS-producing LAB. The TPA investigation showed that cheese produced with EPS-forming LAB was significantly softer than the cheese made without EPS. Furthermore, fat-reduced cheddar cheese produced with the EPS producing strain *Lactococcus lactis* ssp. *cremoris* had the same properties as the full-fat cheese (Awad, Hassan, & Muthukumarappan, 2005). In contrast, a study by Dertli et al. (2016), which investigated the influence of EPS-producing LAB on a Turkish-type fermented sausage (sucuk), showed that sausages produced with an EPS-forming starter culture were harder, tougher, and less adhesive than the product without EPS. These results indicate that the application of *in situ* EPS-forming LAB could lead to different effects in different products. In the present study, the EPS production was found to be high enough to achieve functional properties leading to a softer texture (decreased hardness) of the products.

3.4 Sensory evaluation

The sensory evaluation was conducted to support the findings of the textural measurements and to investigate the effect of the lower pH value on the taste of the final product. In Figure 5, the results of the sensory evaluation of 20 untrained panelists are illustrated. The evaluation showed that the sausages produced with the EPS-producing LAB were tendentially rated higher than 5 (which corresponds to the control sample) for the attributes of spreadability and mouthfeel, except for *L. curvatus* 1.1928 in the first experiment. However, in contrast to the TPA, the sensory results for sausages produced with EPS-forming LAB were not significantly different to the control sample ($P > 0.05$). The taste of the samples was rated the same as for the control, which is a very important result with regard to consumer acceptance. For example, Güler-Akin, Serdar Akin, and Korkmaz (2009) investigated the sensory properties of fat-reduced stirred yoghurt produced with an EPS-producing LAB and compared it to a yoghurt produced with a non-EPS-forming strain. The yoghurt with EPS was disliked compared to the control, and this was, according to the authors,

due to differences in the metabolites; however, the mouthfeel was rated better compared to the control. In contrast, in this study no differences were seen for the sausages produced with or without an EPS-producing strain. Furthermore, Juvonen et al. (2015) produced pureed carrots with and without EPS-producing strains. The sensory evaluation showed that different strains can lead to different results with regard to the sensory properties. Hence, one has to consider that the sensory results could differ for the same product based on the LAB used.

4. PROPOSED MECHANISM

The application of EPS-producing LAB to reduce the pH value of spreadable raw fermented onion mettwurst with the aim of increasing the spreadability and, possibly the safety of the product in the future, seems to be a promising approach.

Figure 6 illustrates the proposed mechanism for the interaction of the charged protein particles, EPS, and fat in raw fermented sausage.

i. At the normal product pH value of 5.5 to 5.6, the protein particles in the sausage are negatively charged (Hamm, 1996) and therefore electrostatic repulsion of the particles prevents a network formation. The product remains spreadable (Figure 6A).
ii. If the pH decreased, it gets closer to the isoelectric point (IEP) of the meat proteins (around 5.0), leading to a reduced net charge directly affecting electrostatic repulsion. This effect, and the overly-low fat content, now leads to the formation of a protein network, and the spreadability of the product is lost (Figure 6B).
iii. The application of EPS-producing LAB leads to the *in situ* production of EPS, resulting in microphase separation between the slightly charged/uncharged protein particles and the uncharged polysaccharides (Grinberg & Tolstoguzov, 1997; van de Velde, de Hoog, Oosterveld, & Tromp, 2015), which leads to a weakened structure and hence a reduced hardness of the sausages as compared to the control (Figure 6C).

This mechanism is supported by the studies of Ayala-Hernandez, Goff, and Corredig (2008) and Hilbig, Loeffler, Herrmann, and Weiss (2019a), who showed that the EPS are located on the surfaces of protein particles.

5. CONCLUSION

The usage of HoPS-producing strains was found to be promising for the production of pH-reduced spreadable raw fermented onion mettwurst (5.1 instead of 5.5 to 5.6) in order to increase the spreadability and possibly the safety of the product in the future. Furthermore, the data obtained made the proposal of a mechanism possible. Moreover, in future studies the structural properties of the EPS (especially molecular weight and polydispersity) and the interactions with proteins should be further investigated.

Moreover, the study showed that different LAB can cause various effects in the meat matrix and that the EPS content formed is also highly strain dependent. Thus, one should be very careful when selecting LAB for prospective industrial applications.

Further research should focus on the safety of the product, because it can now be manufactured with a reduced pH value. Therefore, challenge tests with foodborne pathogens including *Listeria monocytogenes* and *Salmonella enteritidis* should be performed. One should also investigate the potential formation of bacteriocins, which could provide another barrier to food pathogens.

AUTHOR CONTRIBUTIONS

Jonas Hilbig designed the study and was responsible for the experiments and the interpretation of results. Myriam Loeffler was involved in the conception of the study, and writing and proofreading of the manuscript. Lisa Hildebrandt performed most of the experiments and Kurt Herrmann supervised the production in the meat pilot plant. Jochen Weiss supervised the project and contributed substantially to the conception and interpretation of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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