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Influence of exopolysaccharide-producing lactic acid bacteria on the spreadability of fat-reduced raw fermented sausages (Teewurst)

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ABSTRACT

This study aims to reduce the high fat content of spreadable raw fermented sausages (Teewurst) through the application of *in–situ* exopolysaccharide (EPS)-forming lactic acid bacteria (LAB). For this reason, sausages with EPS-forming LAB and different fat contents (20–40% added belly fat) were produced and compared to control products without an EPS-forming culture (*L. sakei* TMW 1.2037). Microbial growth and pH were monitored during processing, the fat (Weibull-Stoldt method) and EPS content (HPLC) of the final products determined, and the products characterized using rheological and texture profile analysis. The fat content of the final products ranged between 17–20%, 25–27%, and 30–33%, respectively. The EPS content of the spreadable raw sausages ranged between 0.08 and 0.30 g/kg for the heteropolysaccharides-producing strain *L. plantarum* TMW 1.1478, and between 0.46 and 1.03 g/kg for the homopolysaccharide (HoPS)-producing strains *L. sakei* TMW 1.411 and *L. curvatus* TMW 1.1928. The latter ones significantly ($p < 0.05$) reduced the hardness of the fat-reduced products and showed lower loss and storage moduli. These results were also supported by the findings of the sensory evaluation where products containing HoPS-forming LAB were rated softer and better spreadable than the corresponding control samples. In addition, the taste of the products was not negatively influenced by the presence of the HoPS-forming LAB.

This study clearly demonstrated that the application of HoPS-producing LAB is a promising approach to reduce the fat content of spreadable raw fermented sausages.

1. Introduction

Nowadays, meat is often associated with a negative impact on human health, which is, among other factors, attributed to its high fat content, since a high dietary fat intake is connected to obesity, hypertension, and cardiovascular disease (Bray, Paeratakul, & Popkin, 2004; Choi et al., 2010; Lurueña-Martínez, Vivar-Quintana, & Revilla, 2004). Therefore, the consumers' demand for healthier or fat-reduced products is a challenge the food industry has to cope with (Arihara, 2006; Biesalski, 2005; Weiss, Gibis, Schuh, & Salminen, 2010). For that reason, several attempts have been made to reduce or replace fat in meat products. For example, Delgado-Pando, Cofrades, Rodríguez-Salas, and Jiménez-Colmenero (2011) investigated the fat replacement in pork liver pâtés (usually 30% fat) with different oils (olive, linseed, and fish oils) and konjac gel (7.5–15%). They demonstrated that samples containing konjac gel had a loosely-structured network which may mimic the normal fat content. Furthermore, other studies also examined the effect of hydrocolloids, including carboxymethylcellulose, locust bean gum, xanthan gum, and rice bran fibers as fat replacers and/or structuring agents in different meat products (Chattong, Apichartsrangkoon, & Bell, 2007; Choi et al., 2009; Choi et al., 2010; Lurueña-Martí;nez et al., 2004). These additives were shown to influence the properties of the meat products in a positive way. However, consumers demand “natural, green-labeled” food (Rownan, 2016). One solution to meet the consumers' demand could be the application of exopolysaccharide (EPS)-forming LAB during meat processing, since these EPS don't have to be labeled, but may influence the quality attributes of the final products provided that enough EPS have been formed. *Lactobacillus* strains produce two types of EPS, homopolysaccharides (HoPS) and heteropolysaccharides (HePS). HoPS are only composed of one monomer of D – glucose or L – fructose, whereas HePS have a more complex structure and are composed of 2–4 types of monosaccharides (Sutherland, 2001). EPS-producing cultures are successfully used in food processing, and have been shown to improve the

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properties of bread, cereals, and dairy products (Marshall & Rawson, 1999; Rühmkorf, Jungkunz, Wagner, & Vogel, 2012; Rühmkorf, Rübsam, et al., 2012; Yilmaz et al., 2015). For example, Perry, McMahon, and Oberg (1997) produced low-fat mozzarella (6% fat) with EPS-producing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. The cheese showed increased moisture content and melt compared to a control produced without these cultures. However, the application of EPS-producing LAB could also be promising for fermented meat products. The traditional German spreadable, raw fermented sausage 'Teewurst' typically consists of pork, beef, and pork fat and has a soft spreadable texture due to its high fat content (30–35%). The structure of the sausage can be described as a protein in fat emulsion in which the meat particles are covered by a layer of fat, which makes the sausage spreadable (Feiner, 2006; Lücke, 2015). Furthermore, it has a low acid content (~pH 5) and loses less than 10% of its original weight during ripening (Dourou et al., 2009; Lücke, 2015). The fermentation (acid production) and drying of the spreadable raw fermented sausage is usually performed at 24 °C and takes 36–48 h, which could be suitable conditions for EPS production (Feiner, 2006). However, only very few attempts have been made to introduce EPS-forming LAB into meat products. For instance, Dertli et al. (2016) investigated the effect of EPS-producing cultures on the texture of the Turkish – type fermented sausage sucuk. The sausages produced with *Lactobacillus plantarum* 162 R and *Leuconostoc mesenteroides* N6 were harder, less adhesive and tougher than the respective control samples.

The hypothesis of the present study is that selected LAB strains are able to produce sufficient amounts of EPS during sausage fermentation, which allow a reduction in the high fat content by keeping spreadability. Therefore, three different EPS-producing *Lactobacillus* strains (two HoPS-— and one HePS-producing LAB) were selected, and products containing one of the EPS-forming bacteria compared to control products containing the non-EPS-forming strain *L. sakei* 1.2037.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

De Man, Rogosa, and Sharpe (MRS) agar, MRS broth (peptone from casein 10.0 g/L, meat extract 10.0 g/L, yeast extract 4.0 g/l; glucose 20.0 g/L, dipotassium hydrogen phosphate 2.0 g/L, Tween® 80 1.0 g/L, di-ammonium 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), Anaerocult® and RSM agar (100 g/L meat extract, 1 g/L Tween 80, 30 g/L nitrite curing salt containing 0.5% $NaNO_2$, 0.6 g/L sodium ascorbate, 3 g/L glucose, lactic acid to adjust to pH 5.8) were purchased from Merck KGaA (Darmstadt, Germany). 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, peptone 5.0 g/L, and yeast extract 2.5 g/L) was obtained from AppliChem GmbH (Darmstadt, Germany). The ion exchange resins Dowex 50WX4 hydrogen form (cationic) and Dowex 66 free base (anionic) were purchased from Sigma-Aldrich Chemie GmbH (Munich, Germany).

2.1.2. Meat and spices

Lean pork meat and pork belly fat were obtained from a local food retail market (MEGA eG, Stuttgart, Germany) and standardized to S II and S IX according to the GEHA meat classification system (Prändl, Fischer, Schmidhofer, & Sinell, 1988). The spices paprika mild, white pepper, nutmeg, cardamom, mono sodium glutamate and the additives ascorbic acid, nitrate curing salt (NCS; containing 0.5% NO_2) and sugar (saccharose and glucose) were purchased from Gewürzmüller GmbH (Ditzingen, Germany).

2.2. Methods

2.2.1. Identification of EPS-forming LAB isolates and growth conditions

To identify bacterial strains suitable for an *in-situ* EPS production in fermented meat products, 77 strains of lactic acid bacteria (LAB) were selected from the in-house strain collection (University of Freising, Munich, Department of Technical Microbiology) and screened for EPS formation. All strains had been originally isolated from cold stored food products (e.g. raw fermented sausage) and belonged to the species of *Lactobacillus (L.) sakei, L. curvatus, L. plantarum, Lactococcus (Lc.) piscium* and *Leuconostoc (Ln.) gelidum.*

Bacterial isolates were recovered from cryo-cultures on modified MRS (mMRS) agar (Stolz, Bocker, Hammes, & Vogel, 1995) supplemented with fructose (5 g/L), glucose (5 g/L) and maltose (10 g/L) by incubation for 48 h at 30 °C or 25 °C (*Leuconostoc* spp.). To screen for EPS production, the strains were transferred to appropriate agar plates allowing for a visual identification of EPS positive strains by characterizing phenotypes. Sucrose-dependent EPS production (i.e. production of homopolysaccharides, HoPS) was detected on mMRS agar supplemented with sucrose (80 g/L). Screening for sucrose independent EPS production was performed on mMRS agar containing increased amounts of yeast extract (10 g/L) and meat extract (10 g/L) as well as glucose, galactose and lactose (20 g/L each), to facilitate a potential formation of heteropolysaccharides (HePS) (Polak-Berecka, Wasko, & Kubik-Komar, 2014). Finally, EPS positive strains were identified after incubation for up to 48 h at 30 °C or 25 °C (*Leuconostoc* spp.). To confirm the EPS formation under mild stress conditions, the strains were additionally screened on RSM agar supplemented with sucrose or glucose/galactose/lactose as described above, and phenotypes identified after incubation at 20 °C for 120 h.

2.2.2. Preparation of the raw fermented sausage

The stock cultures of the *Lactobacillus* strains were inoculated in 60 mL MRS broth for 24 h at 30 °C. After incubation, the cultures were centrifuged (Z32HK, HERMLE Labortechnik GmbH, Wehingen, Germany) at 5000 rpm for 10 min at 20 °C. Afterwards, the MRS broth was discarded to ensure the taste of the sausage not being negatively influenced and the pellet (LAB biomass) then resuspended in 30 mL peptone water (16 g/L peptone), which was used for sausage production. For each strain, three 5 kg batches containing either 20% (w/w), 30% (w/w) or 40% (w/w) added pork belly fat were produced. Control samples were produced for every strain and fat content. The chilled lean pork meat (S II) and pork belly fat (S IX) (raw material used from different animals to account for differences in the pork meat quality) were minced with a 2 mm hole plate in a meat grinder (Type W-114, Maschinenfabrik Seydelmann KG, Stuttgart, Germany) and placed into a precooled bowl chopper (K20 Ras 90181–1, Maschinenfabrik Seydelmann KG, Stuttgart, Germany). After mixing the meat and fat, the spices and the respective LAB inoculum (~10^6 CFU/g) were added. For the HePS-producing strain *L. plantarum* 1.1478, glucose (5 g/kg) and for the HoPS producing strains, saccharose (5 g/kg) was used as a substrate due to the different metabolic pathways in which they produce the EPS. The meat batter was chopped at 3000 rpm until an even distribution of the ingredients was achieved. Nitrite curing salt (26 g/kg) was added at this point and the mixture then chopped again at 1500 rpm. The meat batter was filled (MWF 591, MADO Patron, Dornhan, Germany) into Nalo cellulose casings with a caliber of 45 (Kalle GmbH, Wiesbaden, Germany) and the weight of each sausage documented. Then the sausages were placed in a smoke chamber (Unigar 1800 BE, Ness & Co. GmbH, Remshalden, Germany) to ferment at a temperature of 24 °C and a relative humidity of 90% for 24 h. Subsequently, cold smoke was applied for 1 h (24 °C) and the sausages were dried for several days at a temperature of 18 °C until a weight loss of 6 ± 0.5% (w/w) was achieved. During the whole production process, the weight and the pH of the sausages were recorded. After reaching a weight loss of ~6%, the sausages were packed and stored at

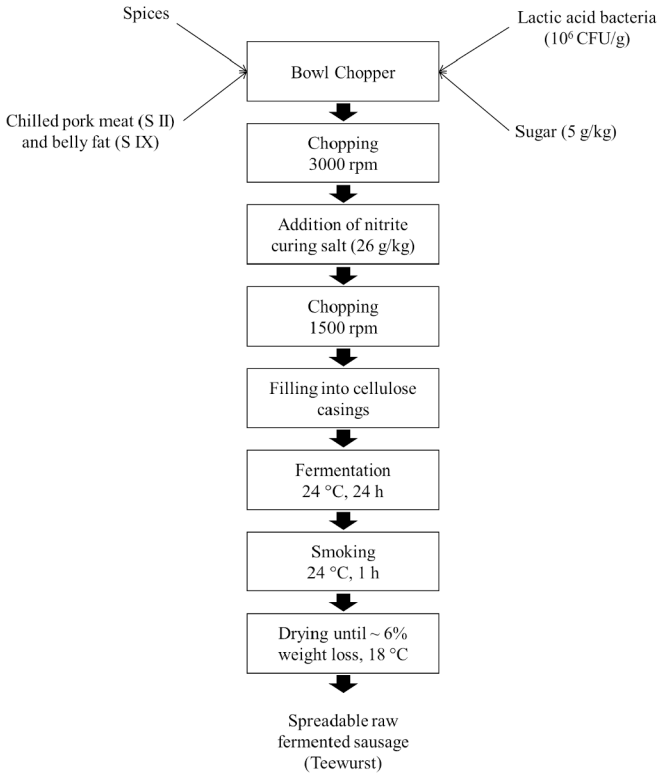

Fig. 1. Process flow chart of the production of fat-reduced raw fermented sausages.

a temperature of 0 °C until analyses were performed. The experiments were repeated using new raw materials and freshly reactivated bacterial cultures but with the same recipe and the same experimental conditions. Fig. 1 shows the process flow chart of the sausage production.

2.2.3. Determination of the fat content

The fat content of the samples was determined by a Soxhlet fat extraction according to the Weibull-Stoldt method (BFR 2004). 10 g of the sausage sample was cooked in 150 mL 4 N HCl for 1 h at a temperature of 115 °C. Then the samples were washed and filtered with hot distilled water until the pH was neutral. The remaining fat samples were stored at 60 °C for at least 12 h. The dried samples were transferred to extraction shells and placed into the Soxhlet extraction chamber (Büchi Labortechnik GmbH, Essen, Germany). Petroleum ether was added to the Soxhlet extraction flask and the samples then extracted for 4 h. The flasks were dried at 60 °C and the weight of the remaining fat was measured.

2.2.4. Microbiological analysis

LAB count of each batch was performed after filling the meat batter into casings, after 24 h and after the sausages reached a weight loss of 6 ± 0.5%. To do this, 10 g of the respective meat sample was mixed with 90 g of peptone in a stomacher bag and homogenized for 60 s with 6 strokes per s using a stomacher (IUL Instrument GmbH, Königswinter, Germany). Dilutions of the samples and the brine were plated with an automated spiral plater (Don Whitley Scientific Limited, West Yorkshire, UK). The bacteria were plated on MRSA plates and on PCA plates. MRSA plates were either stored under anaerobic- (anaerobic atmosphere was generated using Anaerocult®) and PCA plates under aerobic conditions for 24–48 h at 30 °C and colonies then automatically counted using Acolyte (Synbiosis, Cambridge, UK).

2.2.5. pH measurement

The pH of the spreadable raw fermented sausages was monitored with a pH-meter (WTW GmbH, Weilheim, Germany) during the processing of the sausage until a weight loss of 6 ± 0.5% was achieved.

2.2.6. Isolation and quantification of EPS

To quantify the amount of EPS present in the samples, a modified method from Dertli et al. (2016) was used. The sausage samples were homogenized in a KitchenAid® food chopper (Whirlpool Corporation, Benton Charter Township, MI, USA) and dried at 105 °C for at least 12 h. After drying, the samples were crushed, and 10 g of the sample was resuspended in 20 mL of ethanol and incubated for 12 h at 4 °C. Thereafter, the samples were centrifuged at 6000 × *g* for 30 min at 4 °C using a Z32HK centrifuge from HERMLE Labortechnik GmbH (Wehingen, Germany) and the pellets, which contained EPS were suspended in 5 mL of distilled water and dissolved under a temperature lower than 50 °C. To precipitate the proteins the sample was mixed with 15 mL trichloroacetic acid (26.66%) to reach a final concentration of 20% and stored on ice for 1 h. Subsequently, the samples were centrifuged at 13000 × *g* for 20 min at 4 °C to remove the proteins from the supernatant. The supernatant was then mixed with 2 vol of ethanol for the precipitation of the polysaccharides (EPS) and stored for 1 day at 4 °C. The stored sample was centrifuged (10000 g) for 10 min at 4 °C and the pellet then dissolved in 0.714 mL ddH_2O and 0.286 perchloric acid (70%) to reach a final concentration of 5% (v/v). The EPS were then hydrolyzed for 6 h at 95 °C in a water bath and centrifuged (13000 g) at 4 °C for 10 min to remove precipitated proteins. The remaining supernatant was then treated and shaken for 3 min with a mixture of a weak anionic (Dowex 66 free base) and a strong cationic ion exchanger (Dowex 50WX4 hydrogen form) (mixture 1:1) to remove salts and other ions from the solution. For the removal of the ion exchanger, the mixture was centrifuged (3000 g) for 5 min. Finally, the solution was 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 (RI) detector at 40 °C. The results for the HoPS-producing strains were compared to a glucose standard curve. For the HePS-producing strains, the results were compared to a glucose, mannose/galactose, and rhamnose standard curve. The sugars were identified in a first prescreening at the Department of Technical Microbiology at the University of Freising in Munich.

2.2.7. Texture profile analysis

For the texture profile analysis (TPA), an Instron Model 3365 Tensile Tester (Instron GmbH, Darmstadt, Germany) was used. 10 cylinders with a height of 1.5 cm and a diameter of 2 cm were stamped out with a metal pipe. The cylinders were compressed to 50% of their original height in a double compression cycle test with a probe 2.5 cm in diameter, at a cross-head speed of 50 mm/min and equipped with a 5 kN load cell. Furthermore, the force-time deformation curves were obtained. The time between the two compression cycles was 20 s. Hardness, cohesiveness, and springiness parameters were calculated using the time versus force curve.

2.2.8. Rheological measurements

The rheological measurements were carried out with an oscillatory rheometer Physica MCR 502 (Anton Paar GmbH, Karlsruhe, Germany), using a rough plate/plate geometry (plate diameter of 25 mm) with a gap of 2 mm and a temperature of 25 °C. Each sample was placed on the rheometer plate and excess material was removed with a spatula. An amplitude-sweep test was performed twice per sample by increasing the deformation from 0.01 to 100% at an angular frequency of 10 rad/s at 25 °C. Hence, the frequency test (angular frequency 0.01–100 rad/s) was carried out 4 times per sample at a strain value of 0.5%. All data was recorded using the application software RheoPlus 32 V3.31 (Anton Paar GmbH, Karlsruhe, Germany).

2.2.9. Sensory evaluation

A sensory evaluation with 20 untrained panelists was performed in order to grade the attributes of hardness/spreadability, mouthfeel/creaminess, and taste of the samples compared to the respective control sausages. The samples were cut into 1 cm thick slices with a diameter of 4 cm. The task was to evaluate the samples from 0 (very firm consistency, or a dislike of the creaminess or taste) to 10 (very soft sausage, preferred creaminess or taste). For the evaluation of the spreadability, a metal knife and plate were handed to the panelists. The samples had to be spread over a distance of 5 cm and were subsequently evaluated by the panelist. The respective control sample was graded with 5 and the samples had to be compared to it (control = 5; > 5 indicates better mouthfeel, softer products, and/or taste). The sensory test was carried out and recorded with the software Fizz Acquisition 2.51 (Biosystems, France) and Fizz Calculations 2.50 (Biosystems, France).

2.3. Statistical analysis

All measurements were at least repeated twice using duplicate samples. Means and standard deviations were calculated from these measurements using Excel (Microsoft, Redmond, WA, USA). The software SPSS (IMB SPSS Statistics 24, IBM, Germany) was used to statistically evaluate the results. A paired *t*-test was performed for the texture profile analysis, fat contents, pH-values, the rheological measurements and the sensory evaluation to investigate significant differences between the samples and the control ($p < 0.05$). Furthermore, a one-way analysis of variance (ANOVA) was performed with a post-hoc Tukey test ($p < 0.05$) to evaluate results gained from the cell counts and the HPLC measurements.

3. Results and discussion

3.1. Microbiology, pH values and EPS quantification

The screening of the 77 LAB strains revealed 23 positive strains, and EPS production was also demonstrated under mild stress conditions on RSM agar (Table 1). Twelve strains displayed a sucrose-dependent EPS production thereby suggesting the formation of glucans or fructans (Monsan et al., 2001). While these EPS displayed a mucoid (non-ropy) character, the sucrose-independent EPS formation was accompanied by a ropy phenotype with all strains, and mainly occurred among the species of *L. plantarum*. Based on the provided results (Table 1) the following strains were chosen for the present study: the HePS-forming strain *L. plantarum* TMW 1.1478 (Prechtl, Wefers, Jakob, & Vogel, 2018b) and the HoPS-forming strains *L. curvatus* TMW 1.1928 as well as *L. sakei* TMW 1.411 (Prechtl, Wefers, Jakob, & Vogel, 2018a), and as the non-EPS producing control strain *L. sakei* TMW 1.2037 (hereafter referred to as *L. plantarum* 1.1478, *L. curvatus* 1.1928, *L. sakei* 1.411, and *L. sakei* 1.2037, respectively).

Depending on the microorganism used, EPS are formed during different growth phases. For instance, *Lactobacillus* strains were found to produce EPS during the exponential growth phase (Sutherland, 2001; van Geel-Schutten, Flesch, Ten Brink, Smith, & Dijkhuizen, 1998). Table 2 illustrates the anaerobic cell counts determined in the various produced sausages. In the repetition of the experiments, the cell counts were quite similar (data not shown). The sausages were incubated with an initial cell concentration of around 10^6 CFU/g meat. During the fermentation, the cell counts increased to 10^7–10^8 CFU/g meat after 24 h, and further to final cell counts between 10^8 and 10^9 CFU/g meat during the following hours of incubation, except for *L. sakei* 1.411, which remained at 10^7 CFU/g meat. Furthermore, the cell counts of the raw material were found to be in the range of 10^3 and 10^4 CFU/g meat, which indicates a good raw material quality (Feiner, 2006).

Lactic acid bacteria need fermentable sugars to grow, so organic acids, mostly lactic acid, are formed during the fermentation (Leroy & De Vuyst, 2004; Stiles & Holzapfel, 1997). Table 3 illustrates the decrease in the pH values during the experiment. The pH values dropped from 5.65 ± 0.15 depending on the raw materials (meat and fat) to values around and slightly below 5. There were small differences between the samples prepared with the EPS-producing strain and the respective control sample of around 0.2 pH units.

The quantification of EPS by HPLC showed differences between the HePS-producing strain *L. plantarum* 1.1478 and the HoPS-producing strains *L. sakei* 1.411 and *L. curvatus* 1.1928 (Table 4). *L. plantarum* 1.1478 (HePS) yielded EPS amounts from 0.08 to 0.30 g/kg whereas *L. sakei* 1.411 and *L. curvatus* 1.1928 (HoPS) produced 0.50 g/kg up to 1.00 g/kg EPS. That the strains are able to produce EPS was shown in a study done by Hilbig, Loeffler, Herrmann, and Weiss (2018) in which EPS contents of cooked ham model systems significantly increased during 24 h of fermentation. The differences between the two groups can be explained by the different EPS - synthesis pathways. HePS are produced intracellularly in an energy intensive way comparable to the cell wall synthesis, whereas HoPS are synthesized extracellularly by glycosyltransferases (De Vuyst & Degeest, 1999; Lin & Chien, 2007; Monsan et al., 2001). HoPS are usually produced to a higher extent than HePS but, due to the complex structure of HePS, lower amounts may be sufficient to influence the properties of a certain product (Sutherland, 2001; Wingender, Neu, & Flemming, 2012). For instance, Korakli, Pavlovic, Gänzle, and Vogel (2003) showed that the production of HoPS by *Lactobacillus sanfranciscensis* LTH 2590 in a sucrose – MRS medium reached up to 40 g/L under optimal growth conditions. Another study done by Bergmaier, Champagne, and Lacroix (2005) reported that

Table 1
Sucrose dependent (left) and independent (right) EPS formation of screened LAB isolates with assessed EPS amounts according to mucoid (sucrose dependent) and ropy (sucrose independent) phenotypes on mMRS and RSM based selection agar. (+ + +) strong, (+ +) medium, (+) weak EPS formation.

Sucrose dependent EPS formation				Sucrose independent EPS formation			
Strain	Species	EPS formation		Strain	Species	EPS formation	
		mMRS	RSM			mMRS	RSM
1.411	*L. sakei*	+ +	+ +	1.1930	*L. sakei*	+	+
1.578	*L. sakei*	+	+	1.1931	*L. sakei*	+	+
1.1936	*L. sakei*	+ + +	+ +	1.4	*L. sakei*	+	+
1.1937	*L. sakei*	+ +	+ +	1.416	*L. plantarum*	+	+
1.440	*L. curvatus*	+ +	+ +	1.1308	*L. plantarum*	+	+ +
1.624	*L. curvatus*	+ + +	+ + +	1.1478	*L. plantarum*	+	+ +
1.50	*L. curvatus*	+ + +	+	1.64	*L. plantarum*	+	+ +
1.51	*L. curvatus*	+ + +	+	1.708	*L. plantarum*	+	+
1.1928	*L. curvatus*	+ + +	+ + +	1.1879	*L. plantarum*	+	+
2.1616	*Ln. gelidum*	+ +	+	1.1953	*L. plantarum*	+	+
2.1619	*Ln. gelidum*	+	+	1.2022	*L. plantarum*	+	+
2.1620	*Ln. gelidum*	+ +	+				

Table 2
Anaerobic cell counts (CFU/g meat) of *L. plantarum* 1.1478, *L. sakei* 1.411, and *L. curvatus* 1.1928 after the production, after 24 h of fermentation and in the product.

Strain	Added fat (%)	*After production of the sausage batter	*After 24 h	*Product
L. plantarum 1.1478	40	$1.21{\cdot}10^{6} \pm 1.52{\cdot}10^{5a}$	$1.02{\cdot}10^{8} \pm 1.18{\cdot}10^{7b}$	$6.07{\cdot}10^{8} \pm 1.81{\cdot}10^{8b}$
	30	$1.59{\cdot}10^{6} \pm 2.36{\cdot}10^{5a}$	$7.10{\cdot}10^{7} \pm 1.04{\cdot}10^{7b}$	$3.50{\cdot}10^{8} \pm 6.30{\cdot}10^{7c}$
	20	$2.88{\cdot}10^{6} \pm 1.82{\cdot}10^{5a}$	$5.12{\cdot}10^{8} \pm 3.64{\cdot}10^{7b}$	$1.02{\cdot}10^{9} \pm 1.13{\cdot}10^{8c}$
L. sakei 1.411	40	$4.83{\cdot}10^{6} \pm 3.35{\cdot}10^{6a}$	$4.85{\cdot}10^{7} \pm 1.44{\cdot}10^{7b}$	$7.75{\cdot}10^{7} \pm 4.73{\cdot}10^{6b}$
	30	$3.27{\cdot}10^{6} \pm 2.71{\cdot}10^{5a}$	$3.75{\cdot}10^{7} \pm 1.59{\cdot}10^{7b}$	$2.60{\cdot}10^{7} \pm 1.49{\cdot}10^{7b}$
	20	$1.17{\cdot}10^{7} \pm 9.46{\cdot}10^{6a}$	$1.95{\cdot}10^{7} \pm 6.81{\cdot}10^{6a}$	$1.45{\cdot}10^{7} \pm 9.15{\cdot}10^{6a}$
L. curvatus 1.1928	40	$2.99{\cdot}10^{6} \pm 1.08{\cdot}10^{5a}$	$3.40{\cdot}10^{7} \pm 1.25{\cdot}10^{7b}$	$4.27{\cdot}10^{8} \pm 1.72{\cdot}10^{8c}$
	30	$2.48{\cdot}10^{6} \pm 3.06{\cdot}10^{5a}$	$4.75{\cdot}10^{7} \pm 6.19{\cdot}10^{6b}$	$1.46{\cdot}10^{8} \pm 9.15{\cdot}10^{6c}$
	20	$3.40{\cdot}10^{6} \pm 3.23{\cdot}10^{5a}$	$7.45{\cdot}10^{7} \pm 7.19{\cdot}10^{6b}$	$1.05{\cdot}10^{8} \pm 1.05{\cdot}10^{7b}$

*Numbers are means ± standard deviation from duplicates, each examined twice (n = 4).
Values with different letters show significant differences ($p < 0.05$) within the line.

Table 3
pH – values of the raw fermented sausages determined at the end of the production. Sausages were produced using either *L. plantarum* 1.1478, *L. sakei* 1.411, *L. curvatus* 1.1928 or *L. sakei* 12037 (control). The table summarizes the results from the first experiment (1.) and the respective repetition (2.) since different raw material was used.

Strain	40% added fat		30% added fat		20% added fat	
		2.	1.	2.	1.	2.
L. plantarum 1.1478	4.75 ± 0.01^{a}	4.98 ± 0.04^{a}	4.84 ± 0.01^{a}	5.07 ± 0.03^{a}	4.97 ± 0.01^{a}	5.03 ± 0.02^{a}
L. sakei 1.2037 (control)	4.71 ± 0.01^{b}	4.79 ± 0.01^{b}	4.74 ± 0.02^{b}	4.90 ± 0.01^{b}	4.87 ± 0.01^{b}	4.95 ± 0.01^{b}
L. sakei 1.411	4.97 ± 0.01^{a}	4.95 ± 0.02^{a}	5.04 ± 0.01^{a}	5.05 ± 0.01^{a}	5.09 ± 0.01^{a}	5.05 ± 0.01^{a}
L. sakei 1.2037 (control)	4.79 ± 0.01^{b}	4.78 ± 0.01^{b}	4.85 ± 0.01^{b}	4.86 ± 0.03^{b}	4.90 ± 0.01^{b}	4.95 ± 0.02^{b}
L. curvatus 1.1928	4.88 ± 0.01^{a}	5.00 ± 0.01^{a}	4.95 ± 0.01^{a}	5.08 ± 0.01^{a}	4.88 ± 0.02^{a}	5.15 ± 0.01^{a}
L. sakei 1.2037 (control)	4.66 ± 0.02^{b}	4.79 ± 0.06^{b}	4.73 ± 0.01^{b}	4.81 ± 0.01^{b}	4.83 ± 0.03^{b}	4.86 ± 0.01^{b}

All numbers are means ± standard deviation examined three times (n = 3).
Values with different letters show significant differences ($p < 0.05$) within the column and within the line.

Table 4
Quantification of the EPS content of sausages produced with *L. plantarum* 1.1478, *L. sakei* 1.411, and *L. curvatus* 1.1928 (control always subtracted).

Strain	Added fat (%)	*EPS concentration (g/kg dry matter)	
		1.	2.
L. plantarum 1.1478	40	0.20 ± 0.02^{ab}	0.27 ± 0.06^{b}
	30	0.19 ± 0.01^{ab}	0.30 ± 0.04^{b}
	20	0.25 ± 0.01^{b}	0.08 ± 0.01^{a}
L. sakei 1.411	40	0.51 ± 0.01^{cde}	0.79 ± 0.01^{hi}
	30	0.46 ± 0.01^{cd}	0.48 ± 0.04^{c}
	20	0.58 ± 0.01^{def}	0.80 ± 0.02^{hi}
L. curvatus 1.1928	40	0.70 ± 0.01^{gh}	0.67 ± 0.01^{fgh}
	30	0.75 ± 0.01^{hi}	0.86 ± 0.01^{i}
	20	0.62 ± 0.01^{efg}	1.03 ± 0.01^{j}

*Numbers are means ± standard deviation from duplicates, each examined two times (n = 4).
Values with different letters show significant differences ($p < 0.05$) within the column and line.

Lactobacillus rhamnosus RW – 9595 M was able to produce 2 g/L HePS under optimal growth conditions in mineral-supplemented whey permeate. As shown in the present experiments, the production of HoPS was higher than for HePS, but both yields were lower compared to values found in current literature; however, a direct comparison is not possible since EPS formation strongly depends on the strain and growth conditions. For instance, the presence of spices and nitrite curing salt (2.6%), as well as the processing/fermentation temperatures of 18–24 °C, influence EPS production in raw fermented sausages. For example Prechtl et al. (2018a) investigated the EPS production of the strain *L. sakei* 1.411 in a modified MRS medium and found that the EPS yield was higher at a lower temperature of 10 °C (6.7 g/L) compared to the optimal growth temperature of 30 °C (1.6 g/L).

3.2. Fat content

The fat content of the product is important for the spreadability of the sausage, because it is a protein in fat emulsion, where the layer of fat in the outer phase covers the meat particles and makes the sausage spreadable (Lücke, 2015). The determined fat contents of the produced sausages are shown in Table 5. The analytical fat contents are lower than the added belly fat. With addition of 40% belly fat, final fat contents between 30 and 33% could be determined; with an addition of 30%, the sausages had fat contents between 24 and 26%; and 20% led to values between 17 and 20%. This is because the meat contains fat and the belly fat contains meat protein. This was also the reason why the meat was classified according to the GEHA system into different groups prior to production. The meat was classified as S II, which means that it contains up to 5% fat, and the belly fat was classified as S IX, which can contain up to 5% of meat protein (Prändl et al., 1988). If the fat content is reduced too much, the proteins can form a network and thus reduce the spreadability of the sausage (Yang, Kim, Choi, & Joo, 2010).

3.3. Texture profile analysis (TPA)

Hydrocolloids are usually added to produce fat-reduced spreadable sausages, but they have to be labeled on the product. For example, Delgado-Pando et al. (2011) investigated the fat replacement by konjac

Table 5
Determined fat content of the raw fermented sausages produced with either *L. plantarum* 1.1478, *L. sakei* 1.411, *L. curvatus* 1.1928 or *L. sakei* 12037 (control). The table summarizes the results from the first experiment (1.) and the respective repetition (2.) since different raw material was used.

Strain	40% added fat		30% added fat		20% added fat	
		2.	1.	2.	1.	2.
L. plantarum 1.1478	33.05 ± 0.68[a]	32.32 ± 0.21[a]	26.33 ± 0.15[a]	26.06 ± 0.12[a]	17.30 ± 0.01[a]	19.17 ± 0.34[a]
L. sakei 1.2037 (control)	31.04 ± 0.13[a]	33.83 ± 0.24[a]	25.91 ± 0.12[b]	26.65 ± 0.17[b]	20.31 ± 0.01[b]	18.89 ± 2.08[a]
L. sakei 1.411	30.67 ± 0.09[a]	31.43 ± 0.04[a]	24.96 ± 0.02[a]	25.52 ± 0.21[a]	17.80 ± 0.28[a]	19.88 ± 0.08[a]
L. sakei 1.2037 (control)	29.03 ± 0.01[b]	32.12 ± 0.28[a]	25.82 ± 2.44[a]	26.19 ± 0.28[a]	18.38 ± 0.02[a]	20.36 ± 0.40[a]
L. curvatus 1.1928	32.03 ± 0.52[a]	31.08 ± 0.29[a]	25.52 ± 0.11[a]	25.12 ± 0.11[a]	20.90 ± 0.40[a]	20.11 ± 0.66[a]
L. sakei 1.2037 (control)	31.56 ± 1.14[a]	31.76 ± 0.12[a]	24.62 ± 3.07[a]	26.51 ± 0.46[a]	20.59 ± 0.05[a]	20.70 ± 0.29[a]

All numbers are means ± standard deviation examined three times (n = 3).
Values with different letters show significant differences ($p < 0.05$) within the column.

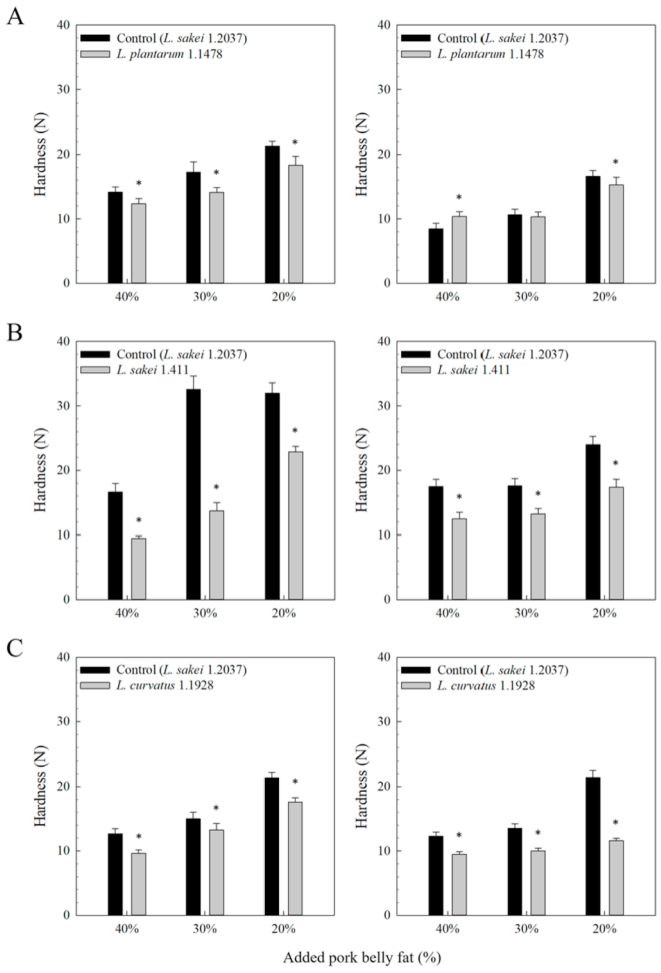

Fig. 2. Hardness of sausages produced with *L. plantarum* 1.1478 (A), *L. sakei* 1.411 (B), or *L. curvatus* 1.1928 (C) and the respective control sausages (*L. sakei* 1.2037) of the first and the second experiment. An asterisk indicates significant differences to control samples ($p < 0.05$).

gels in spreadable pork liver pâtés. The products showed decreased penetration forces and gel strengths. Whereas the addition of 10% hydrated oatmeal was shown to reduce the hardness of low – fat sausages produced with beef, pork, and chicken meat (Yang et al., 2010). Introducing EPS-forming LAB it is necessary to determine the texture, appearance and flavor of food, because they are important sensory factors that need to be accepted by consumers (Pons & Fiszman, 1996).

The values for springiness and cohesiveness showed no differences between the control and the samples produced with EPS-forming LAB. The values decreased with increasing fat content (Data not shown). In Fig. 2, the results of the hardness of the TPA are illustrated. The hardness of the samples prepared with *L. plantarum* 1.1478 had significantly lower values ($p < 0.05$) compared to the respective control sample at all fat contents. Whereas, no significant differences were found in the repetition for the samples prepared with *L. plantarum* 1.1478 and added fat values of 20 and 30%. At 40%, the control sausage even showed a significantly lower hardness ($p < 0.05$) compared to sausages prepared with *L. plantarum* 1.1478 (Fig. 2A). This could be due to the fact that *L. plantarum* 1.1478 is a HePS-forming strain producing less polysaccharides than the HoPS-forming strains and, furthermore, instabilities in the HePS production were reported (Bergmaier et al., 2005; Degeest, Vaningelgem, & De Vuyst, 2001; Korakli et al., 2003). The first statement is supported by the quantification of the produced EPS since the HePS-forming strain *L. plantarum* 1.1478 was found to produce less EPS than the HoPS-producing strains *L. sakei* 1.411 and *L. curvatus* 1.1928 (Table 4). Although lower amounts of HePS are usually needed to cause structural changes, the amount present in the sausage seems not to be high enough for the expected changes. In contrast, the hardness of the samples produced with the two HoPS-forming strains showed significantly lower values ($p < 0.05$) than the respective control samples in both experiments (Fig. 2B and C). The hardness of the samples with 30% added belly fat was at the same level as the control sample, with 40% in the first experiments. Furthermore, the hardness of the samples in the repetition, at 20%, was comparable to the control samples with 40% added belly fat. In contrast to the findings in the present study, Dertli et al. (2016) reported that sucuk (Turkish style raw fermented sausage) produced with the EPS-producing strains *Lactobacillus plantarum* 162 R and *Leuconostoc mesenteroides* N6 showed a harder, less adhesive, and tougher texture. These results demonstrate that EPS production and its influence on product properties strongly depends on the product matrix, bacterial strain, and processing conditions used.

3.4. Rheology

The results of the TPA were supported by the rheological investigations. The results of the frequency sweeps of the different samples with 30% added belly fat are illustrated in Fig. 3. The elastic properties dominated in all samples, which is indicated by the storage modulus (G′) being larger than the loss modulus (G″). In row A, the results of the first and the second experiment of the samples prepared with *L. plantarum* 1.1478 are shown. The storage and loss moduli of the samples with EPS-producing LAB were always lower than the control sample. But for the first experiment, the values showed no significant

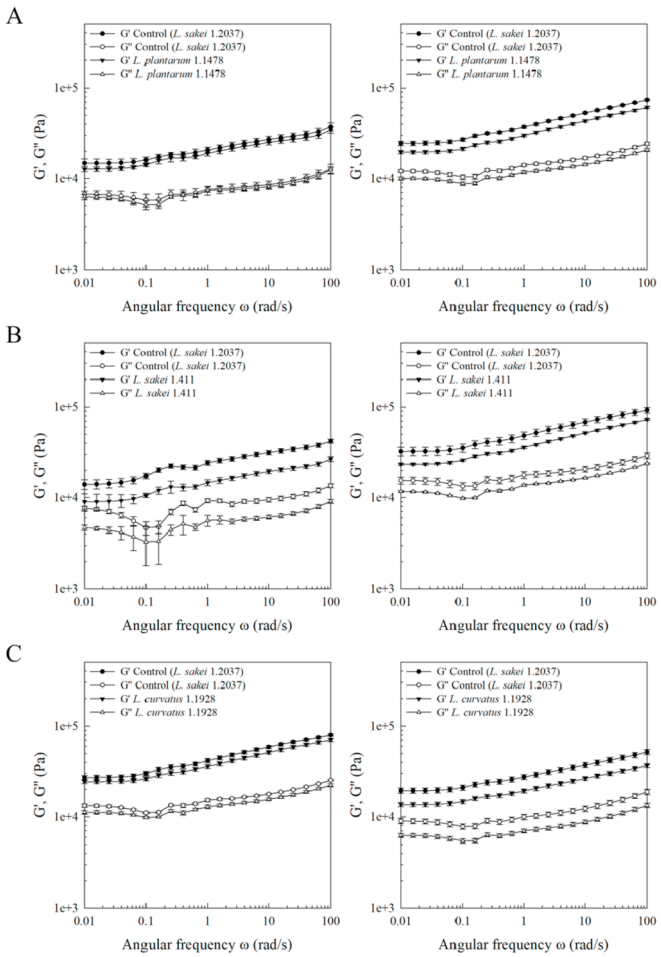

Fig. 3. Frequency sweeps of the sausages produced with 30% added belly fat and with the starter cultures *L. plantarum* 1.1478 (A), *L. sakei* 1.411 (B), and *L. curvatus* 1.1928 (C) compared with the respective control sausages (*L. sakei* 1.2037) of the first and the second experiment.

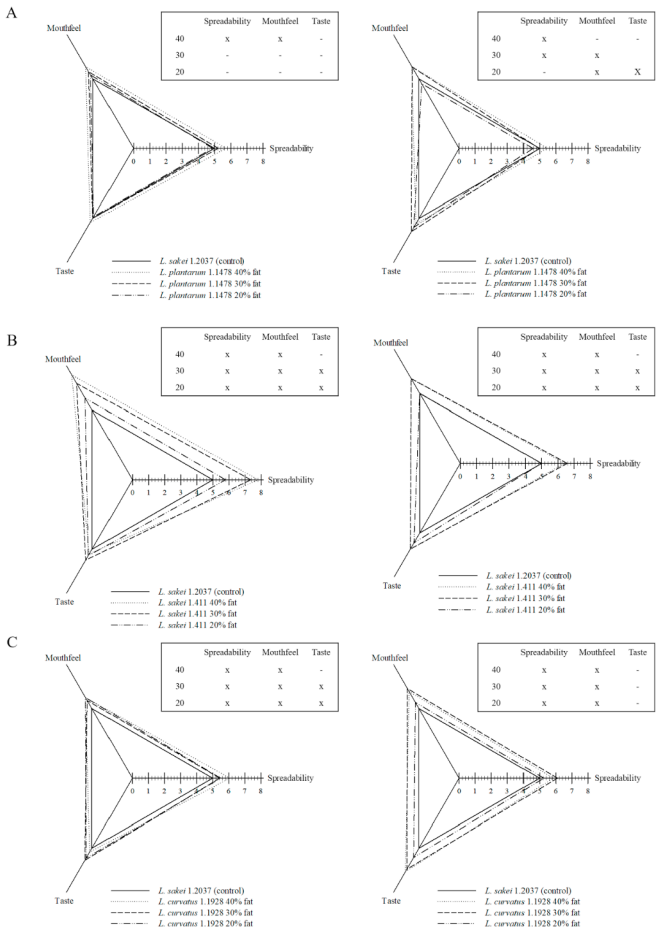

Fig. 4. Sensory evaluation of the sausages produced with *L. plantarum* 1.1478, *L. sakei* 1.411, and *L. curvatus* 1.1928 compared with the respective control sausages (*L. sakei* 1.2037) of the first and the second experiment. In the boxes the statistical evaluation is shown x indicate significant differences compared to the respective control sample ($p < 0.05$). N = 20 panelists.

difference between the control and the samples with EPS-producing LAB ($p > 0.05$), whereas in the repetition, significant differences between the samples were found ($p < 0.05$), which indicates a softer structure. The sausages prepared with the HoPS-producing LAB *L. sakei* 1.411 and *L. curvatus* 1.1928 led to significantly ($p < 0.05$) lower storage and loss moduli in both experiments than the respective control samples, which shows that the structure of the samples was softer compared to the control. Low-fat pork liver pâtés produced with konjac gel showed the same behavior. Both moduli were lower than the control sample in a frequency sweep test (Delgado-Pando, Cofrades, Ruiz-Capillas, Triki, & Jiménez-Colmenero, 2012). Furthermore, Chattong et al. (2007) investigated the influence of 1% xanthan gum on Yor, an ostrich meat product from Thailand, and this treatment reduced the storage and loss moduli compared to the control samples. However, as indicated before, in contrast to hydrocolloids that are added during production, *in-situ*-formed EPS do not have to be labeled, so that the application of such LAB could be a promising approach to meet consumers' demand for "more natural" food products.

3.5. Sensory evaluation

To support the findings of the textural measurements (TPA and rheology) and to investigate whether the used LAB influence the taste and texture of the products, a sensory evaluation with 20 untrained panelists was performed. The results of the sensory panel are presented in Fig. 4. The attribute hardness was graded higher than 5 in all cases (which corresponds to the control sample), except for the sample prepared with the HePS-producing strain *L. plantarum* 1.1478 and 20% added belly fat. Compared to the other sausages that were prepared with the HoPS-producing strains *L. sakei* 1.411 and *L. curvatus* 1.1928, the hardness was tendentially graded lower for *L. plantarum* 1.1478 which is in accordance with the TPA and rheological results. The same correlation was found with respect to samples' spreadability which was significantly better ($p < 0.05$) for sausages containing one of the HoPS-forming strains (independent of the fat content used). The improvement in spreadability could also be confirmed through the interpretation of force-distance curves gained from the TPA measurements (Appendix AP1 A) and the corresponding shear stress curves (Appendix AP1 B). Furthermore, the samples with EPS-producing LAB had a better mouthfeel. The taste of the product is very important for consumer acceptance, and it was shown that the samples tasted as good as, or even better than, the control and moreover, no off -flavor was detected by the panelists. LAB primarily contribute to flavor generation in raw fermented sausages due to the production of large amounts of lactic acid and fewer amounts of acetic acid (Leroy & De Vuyst, 2004). Typical other raw fermented sausage aroma compounds are usually formed by staphylococci (Leroy, Verluyten, & De Vuyst, 2006). Tomaschunas et al. (2013) investigated the influence of inulin and

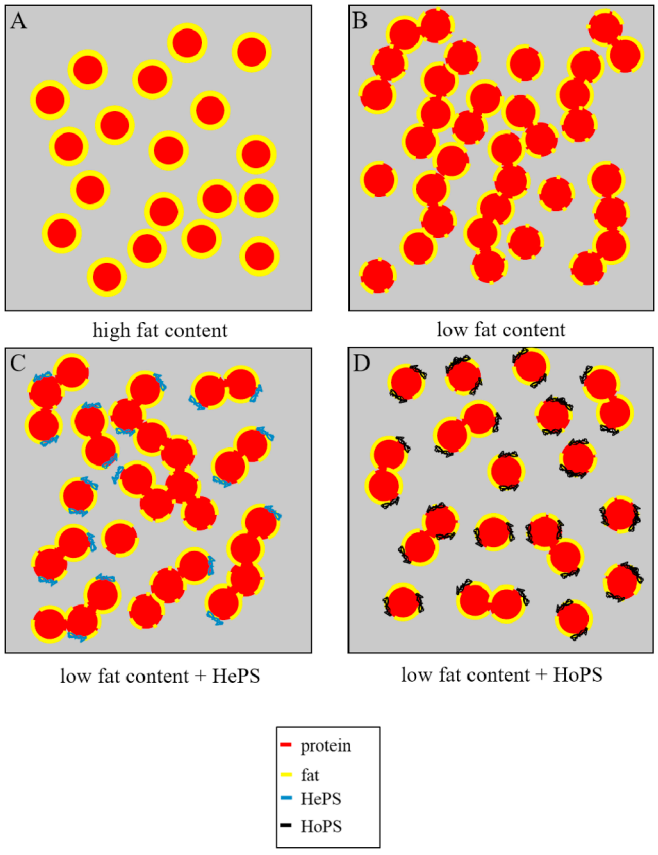

Fig. 5. Proposed mechanism of the interactions between protein, fat, and EPS in the produced sausages. (Red = protein, yellow = fat and black = EPS).

citrus fiber as fat replacers on the sensory impact of fat reduced pork Lyon-style sausage and fat-reduced liver sausage. The sensory impact of the products was changed by the addition of the hydrocolloids and fat reduction; the meat flavor decreased for the Lyon-style sausage. Furthermore, the creaminess of the liver sausage was decreased by adding the hydrocolloids.

4. Proposed mechanism

The application of EPS-producing LAB to reduce the fat content of spreadable raw fermented sausage seems to be a promising approach.

Fig. 5 illustrates the proposed mechanism for the interaction of EPS, protein and fat in the fermented sausage.

i. In the full-fat sausage (40% added belly fat), the proteins are fully covered by fat and the sausage has a good spreadability (Fig. 5A).
ii. If the fat content is reduced, the proteins are no longer fully covered by fat and protein cross-links can be formed (Fig. 5B), reducing the spreadability of the product (Feiner, 2006; Yang et al., 2010).
iii. The application of EPS-forming LAB leads to the production of certain amounts of EPS (HePS or HoPS) that can cover the fat-free areas on the protein particles thereby maintaining the spreadability. HePS are branched and, therefore, a lower amount is often sufficient to cause structural changes as compared to HoPS-forming strains. In the present study, however, the amount of produced HePS was found to be too low to cause consistently better results, indicating that the proteins could not be fully covered and/or linked (Fig. 5C). In contrast, sufficient amounts of HoPS were produced to cover the fat-free areas of the proteins and maintain the spreadability (Fig. 5D).

This mechanism is supported by a study conducted by Ayala-Hernandez, Goff, and Corredig (2008) who immobilized dairy protein particles on an observation surface and performed a washing step after the fermentation to remove all non-interacting material from the surface. The samples were then examined using scanning electron microscopy. The authors concluded their study with the finding that bacteria cells bind themselves to the protein particle via EPS strands.

5. Conclusion

Using HoPS-forming strains, spreadable raw fermented sausages with an added fat content of only 20% could be produced that were in the acceptable range (spreadability, texture, creaminess, taste) for the sensory panel. TPA and rheological investigations proved to be suitable methods for the determination of the textural properties of the sausages and, combined with a quantification of the EPS by HPLC, a concentration – function relationship could be proposed (mechanism).

This study also showed that careful consideration is needed to determine which strains are appropriate to reduce fat in raw fermented sausages, with the HoPS- forming strains *L. sakei* 1.411 and *L. curvatus* 1.1928 being more suitable than the HePS-forming strain *L. plantarum* 1.1478.

Overall, the addition of the EPS-producing LAB could increase consumer acceptance of the product, not only because it contains less fat, but also since the EPS do not need to be labeled on the package.

Further research could focus on the initial sugar content of the meat batter that directly affects the product's pH (fermentation). Using EPS-forming LAB may have the potential to produce spreadable raw fermented sausages that are spreadable at a lower pH, contributing to the shelf life of the product. Additionally, mixtures of saccharose and glucose could be examined, to see if the HoPS-forming strains are able to produce a higher amount of EPS.

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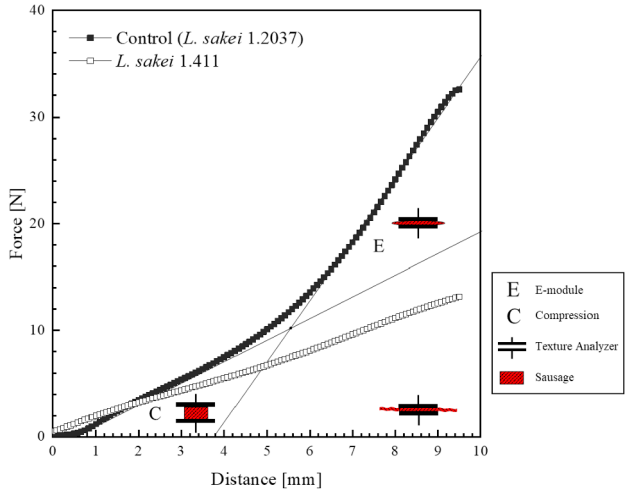
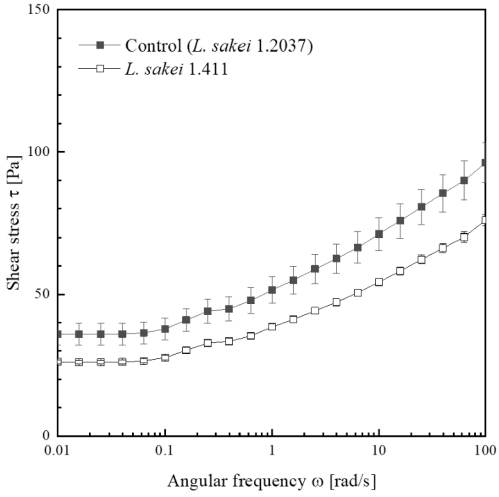
Appendix

AP1 A illustrates the force-distance curve of sausages produced with *L. sakei* 1.411 and 30% added bally fat compared to the respective control sample and highlights the behavior during compression. At the beginning both samples behaved quite similar but at a higher degree of compression the sample produced with the EPS-forming strain *L. sakei* 1.411 flowed out of the texture analyzer indicating spreadability. Compared to that the control sample showed an elasticity module (E) indicating that the sausage is not spreadable. In **AP1 B** the corresponding graph of the shear stress τ is illustrated and was found to be lower for sausages produced with the EPS-forming strain *L. sakei* 1.411, which corresponds well to the shown behavior in **AP1 A**.

A



B



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