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Application of exopolysaccharide-forming lactic acid bacteria in cooked ham model systems

Jonas Hilbig, Myriam Loeffler, Kurt Herrmann, Jochen Weiss*

Department of Food Physics and Meat Science, Institute of Food Science and Biotechnology, University of Hohenheim, Garbenstrasse 21/25, 70599 Stuttgart, Germany

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ABSTRACT

The meat industry often applies hydrocolloids (not label-free) to improve quality attributes of meat products including reconstructed cooked ham. A new approach to improve product quality could be the usage of *in-situ* Exopolysaccharide (EPS)- forming lactic acid bacteria (LAB) provided that these strains are able to produce EPS in meat matrices under typical processing conditions *(here: cooked ham)*. Two homopolysaccharide- (*L. curvatus* TMW 1.624 and *L. sakei* TMW 1.411; $10^{6}\frac{CFU}{mL}$) and heteropolysaccharide-forming LABs (*L. plantarum* TMW 1.1478 and TMW 1.25; $10^{6}\frac{CFU}{mL}$) were hence examined for EPS formation in a cooked ham model system consisting of minced pork topside (< 2% fat) and 16.67% brine containing either 0.5% sucrose or dextrose. Samples were stored for 48 h at either 2 °C to simulate typical tumbling conditions, or at 15 °C to examine *in-situ* EPS production under reduced stress conditions. Microbial growth behavior and pH development (48 h) were monitored and EPS qualitatively as well as semi-quantitatively analyzed using both confocal laser scanning microscopy and MATLAB enabling a better comparison of the investigated strains. All LAB were able to tolerate the suboptimal growth conditions in the cooked ham model systems (2 °C, 1.92% nitrite curing salt) and were found to already produce EPS within 10 h of storage at 2° and 15 °C. EPS amounts detected after 24 h of incubation were significantly ($p < 0.05$) higher than those determined after sample preparation. EPS were found to be predominately located at the outer edge of meat proteins. All investigated strains seem to be promising for prospective studies in cooked ham.

1. Introduction

Exopolysaccharides (EPS) are defined as extracellular polymeric substances of biological origin (bacteria, archaea and eukaryote) that participate in the formation of microbiological aggregates (Wingender, Neu, & Flemming, 2012). As in this study, the term EPS is often used as an abbreviation for “exopolysaccharides”; however, other compounds can appear in significant amounts such as proteins, nucleic acids, and amphiphilic compounds including phospholipids and glycoproteins (Czaczyk & Myszka, 2007). Most microbial EPS are highly soluble in water or in diluted salt solutions and can be divided into the two different groups of homopolysaccharides (HoPS) and heteropolysaccharides (HePS). HoPS are generally synthesized out of sucrose whereas HePS are synthesized out of a variety of different substrates. EPS form a three-dimensional network that may impact the structure and texture of food products. These structures are often formed under suboptimal, or so-called “stress conditions”, in order to protect the microorganisms (Czaczyk & Myszka, 2007; Sutherland, 2001; Wingender et al., 2012). Many microorganisms that are generally recognized as safe (GRAS) have the ability to produce EPS, including lactic acid bacteria (LAB).

In the meat industry, carrageenan (Prabhu & Sebranek, 1997) and starches (Resconi et al., 2016) are often applied to change the texture properties (*e.g.* spreadability) or water-holding capacity of products, including restructured cooked ham (Motzer, Carpenter, Reynolds, & Lyon, 1998). However, these polysaccharides have to be labeled as additives (Brewer, 2012; Schuh et al., 2013). The application of *in-situ* EPS-producing microorganisms could be one possibility to meet the consumer demand for less-labeled, or even “green-labeled” products, since these polysaccharides are produced *in-situ* during processing and thus don't have to be labeled. In appropriate amounts, *in-situ*-produced EPS were already found to improve the properties of products like yoghurts (Amatayakul, Halmos, Sherkat, & Shah, 2006; Bouzar, Cerning, & Desmazeaud, 1997; Purwandari, Shah, & Vasiljevic, 2007) or gluten-free breads (Rühmkorf, Jungkunz, Wagner, & Vogel, 2012).

However, the application of EPS-forming bacteria to improve properties of meat products is a very new field. Only few attempts have been made to introduce EPS-producing Lactobacillus strains in meat

* Corresponding author.
E-mail address: j.weiss@uni-hohenheim.de (J. Weiss).

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products. For instance, Dertli et al. (2016) produced sucuk (Turkish-type fermented sausage) with EPS-forming Lactobacillus strains, leading to harder and less adhesive products compared to the control samples. Furthermore, the study of Prechtl, Wefers, Jakob, and Vogel (2018) showed the ability of *L. sakei* 1.411 to produce EPS even under cold and salt stress conditions. Based on these results LAB strains may also have the ability to tolerate "stress conditions" that exist during cooked ham manufacturing (high salt content 1.92% and low storage temperatures), while producing *in-situ* EPS. To prove this hypothesis four *Lactobacillus* strains (homo- and heteropolysaccharide-forming LAB) were selected and investigated for their potential to produce EPS in a cooked ham model system under typical tumbling conditions.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

MRS agar, MRS broth, and Anaerocult® were purchased from Merck KGaA (Darmstadt, Germany) while Rogosa agar and peptone water were purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Plate Count agar (PCA) was obtained from AppliChem GmbH (Darmstadt, Germany). Calcofluor White Stain and Concanavalin A were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

2.1.2. Microorganisms

The cultures were obtained from the Technical University of Munich (Dept. of Technical Microbiology, Freising, Germany) and were selected out of 77 strains of different lactic acid bacteria species (*Lactobacillus plantarum, Lactobacillus sakei, Lactobacillus curvatus, Leuconostoc gelidum, Lactococcus piscium*) that were initially screened for their potential to produce homo- or heteropolysaccharides. The strains that were selected to be used in the cooked ham model systems are *L. curvatus* TMW 1.624, *L. plantarum* TMW 1.1478, *L. plantarum* TMW 1.25, and *L. sakei* TMW 1.411 (hereafter referred to as *L. curvatus* 1.624, *L. plantarum* 1.1478, *L. plantarum* 1.25, and *L. sakei* 1.411, respectively). All strains were maintained at −80 °C in 20 wt% glycerol and re-activated on MRS plates prior to the experiments.

2.1.3. Meat and brine ingredients

Lean pork meat (topside) with a fat content < 2% was purchased at MEGA das Fach-Zentrum für die Metzgerei und Gastronomie eG (Stuttgart, Germany).

The brine used was composed of 87.9% water, 11.5% nitrite curing salt (NCS), 0.5% sucrose for *L. curvatus* 1.624 and *L. sakei* 1.411 (HoPS-former), or 0.5% dextrose for *L. plantarum* 1.1478 and *L. plantarum* 1.25 (HePS-former), as well as 0.1% ascorbate.

2.2. Brine and cooked ham model systems preparation

2.2.1. Brine preparation

L. plantarum 1.1478 and 1.25 were diluted from $10^{9\frac{CFU}{mL}}$ to $10^{8\frac{CFU}{mL}}$, in peptone water, whereas *L. curvatus* 1.624 and *L. sakei* 1.411 could be used without further preparation. Afterwards, 11.4% of the water needed to prepare the brine was replaced by the diluted and undiluted culture solution of the respective strain ($10^{8\frac{CFU}{mL}}$), which resulted in an initial bacteria concentration of $\sim 10^{6\frac{CFU}{mL}}$.

To investigate whether *L. curvatus* 1.624 and *L. sakei* 1.411 are also able to produce HePS, a plausibility control was conducted in which the two strains were also examined with dextrose instead of sucrose.

2.2.2. Preparation of the cooked ham model systems

Lean pork meat (< 2% fat) was minced in a meat grinder (Maschinenfabrik Seydelmann KG, Stuttgart, Germany) to a size of 5 mm. The inoculated brine with the respective strain was added to the minced meat. The components were then mixed in a KitchenAid stand mixer (KitchenAid Artisan®, Whirlpool Corporation, Benton Charter Township, MI, USA) for 3 min on stage 1. After 1 and 2 min the mixer was stopped and the meat-brine-mixture (hereafter referred to as cooked ham model system) was removed from the wall of the bowl. Afterwards, the respective cooked ham model system was portioned and samples of 30–35 g were packaged into vacuum bags and subsequently evacuated to 0.02 bar pressure. Half of the samples were stored at 2 °C and half of them at 15 °C in either a cold storage or a ripening room.

2.2.3. Preparation of cooked ham model systems for proof of concept

To prove whether the CLSM method is suitable to stain and then qualitatively analyze *in-situ*-produced EPS in the cooked ham model systems, the strains were examined without the addition of sugars, and the sugars were examined without the addition of the LAB strains, respectively. Without the addition of sugars, the brine composition changed to 88.4% water, 11.5% NCS, and 0.1% ascorbate. The brine composition with sugars, but without the addition of bacteria, was 87.9% water, 11.5% NCS, 0.1% ascorbate, and 0.5% sucrose or 0.5% dextrose. The samples were prepared and stored as described in Section 2.2.2.

2.3. Microbiology

Viable cell counts were determined after 0, 6, 10, 16, 24, 30, and 48 h (for proof of concept after 0 and 24 h). The process consisted of 10 g of the respective cooked ham model sample being mixed with 90 g of peptone water in a stomacher bag and homogenized with a stomacher (Masticator Laborhomogenisator, IUL Instrument GmbH, Königswinter, Germany). Dilutions of the samples were plated with an automated spiral plater (Whitley Automatic Spiral Plater, Don Whitley Scientific Limited, West Yorkshire, UK). *L. curvatus* 1.624 was plated on Rogosa agar while the other three bacteria were plated on MRS agar. The minced raw meat was also investigated at 0 h of storage for total viable counts on PCA and MRS agar plates. Plates were either stored under anaerobic (MRS agar) or aerobic (PCA) conditions for 24–48 h at 30 °C and colonies then automatically counted using a plate counter (aCOlyte Synbiosis, model no.: 7510/SYN, Cambridge, UK).

Each experiment was repeated at least 2 times using the same raw material (divided in 2 portions), but with 2 independently inoculated cultures of the same strain. 4 bags/model system (2* 2 °C and 2* 15 °C) and LAB strain were thus analyzed at each sampling time.

Results are shown as mean values ± standard deviation.

2.4. pH measurement

The pH values of the minced raw meat samples were measured after 0 h, whereas the model systems which were stored at 2 °C and 15 °C were analyzed after 0, 24, and 48 h (for proof of concept after 0 and 24 h) using a pH meter (WTW Microprocessor pH Meter, Germany). Results are shown as mean values ± standard deviation.

2.5. CLSM staining of samples

The staining of the samples was done according to Hassan, Frank, and Qvist (2002), who investigated the distribution of EPS in milk and in stirred and unstirred fermented milk, using Concanavalin A and Calcofluor White Stain.

For the CLSM (Nikon Eclipse-Ti inverse microscope, Nikon, Düsseldorf, Germany) investigation, both *in-situ*-formed EPS and meat proteins were stained. Preliminary experiments, in which fat (Nile red), proteins (Calcofluor White Stain) and EPS (Concanavalin A) were stained, showed that the EPS are localized around the proteins (not shown). Because of that, and the fact that < 2% fat is present in the

cooked ham model systems, only proteins and EPS were subsequently stained.

To guarantee that Concanavalin A (1:20 diluted with 10 mmol phosphate buffer; pH 6) is suitable to stain EPS in meat, a preliminary experiment was performed in which different concentrations of dextran (from *Leuconostoc* spp. Mr. ~6000; 0–1%) were mixed with raw meat and afterwards stained and analyzed as subsequently described. It could be shown that Concanavalin A is generally suitable for EPS staining (Supplement data **Table SD1** and **Fig. SD1**). Calcofluor White Stain was used as provided.

The presence of *in-situ*-produced EPS was analyzed after 0, 10, 24, and 48 h (for proof of concept after 0 and 24 h) of storage at 2 °C and 15 °C, by gently opening the packages and stamping a sample out of the cooked ham model system with a sterile metal pipe (diameter 1.5 cm) and putting them on a sample carrier. To stain the EPS, 10 µL of Concanavalin A was used, and the samples then kept darkened in the fridge at 4 °C for approximately 30 min. Afterwards, the samples were stained with 10 µL Calcofluor White Stain and covered with a coverslip. An argon laser at 488 nm and a red helium-neon laser at 638 nm were used for the excitation of EPS and proteins. A 60-fold magnification lens with immersion oil was used to examine the stained meat samples. At least 5 pictures were taken at 5 different spots of each sample in order to gain a good overview of the distribution of EPS in the cooked ham model samples. Scales were inserted using the software ImageJ (Version 1.4.3.67, National Institutes of Health, Bethesda, MD, USA) after creating an RGB picture out of the different channels.

2.6. Image analysis

The CLSM pictures were further analyzed according to a slightly modified code/procedure developed by Bosse, Gibis, Schmidt, and Weiss (2015) using the program MATLAB (Version R2014b, The MathWorks Inc., Natick, MA, United States). The procedure is demonstrated and explained in Fig. 1. At first, only the green channel was saved (tif) which corresponds to the stained EPS. Afterwards, the pictures were analyzed with a script in MATLAB that automatically transformed the green channel of the picture to a black and white picture. Too-small pixels were excluded and only pixels with a threshold over 0.08 were counted. This process was necessary to remove noise. In the resulting picture, the percentage of the green area compared to the whole area was calculated using Eq. 1:

$$\text{Green area (\%)} = \frac{\text{green area (pixels)}}{\text{whole area (pixels)}} \cdot 100\% \quad (1)$$

2.7. Statistical analysis

The measurements for the growth kinetics in the cooked ham model systems were repeated two times using duplicate samples and the pH values were recorded three times using duplicate samples. Five CLSM pictures were taken using duplicate samples. Means and standard deviations were calculated using Excel 2013 (Microsoft, Redmond, WA, USA). The software SPSS (IMB SPSS Statistics 24, IBM, Germany) was used to statistically evaluate the results. A one-way analysis of variance (ANOVA) was performed with a post-hoc Duncan test ($p < .05$) to evaluate results gained from the pH measurements and image analysis.

3. Results and discussion

3.1. Qualitative EPS-analysis: proof of concept

To verify that EPS detection using CLSM is an appropriate method to qualitatively analyze EPS in minced meat and cooked ham model systems, a proof of concept was performed assuming that EPS can only be

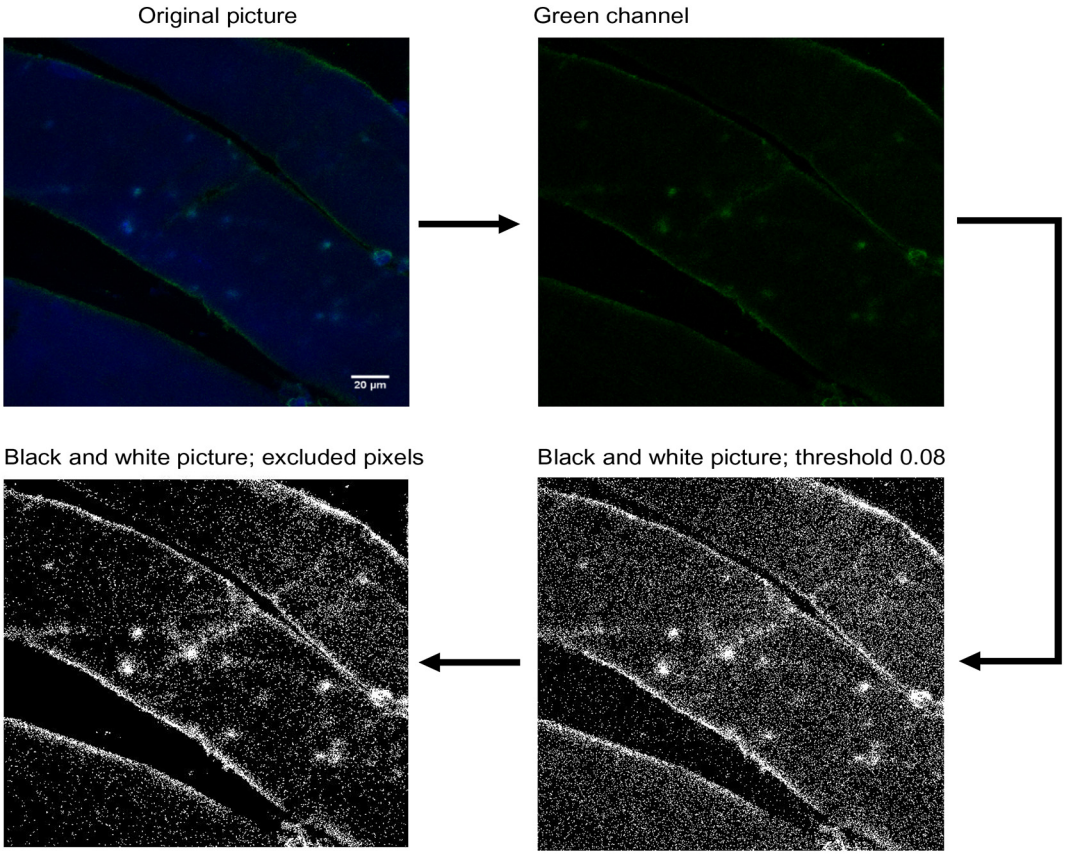

Fig. 1. CLSM image analysis.

Table 1
pH values of model systems containing 1.92% nitrite curing salt, 0.03% ascorbate, and 0.167% sucrose or dextrose but no Lactobacillus strains. Or model systems containing either *L. curvatus* 1.624, *L. sakei* 1.411, *L. plantarum* 1.1478, or *L. plantarum* 1.25, but no sugar.

Sample	Temperature (°C)	pH value[a]	
		0 h	24 h
Dextrose	2	5.53 ± 0.00	5.57 ± 0.01
Dextrose	15	5.53 ± 0.00	5.57 ± 0.01
Sucrose	2	5.50 ± 0.01	5.56 ± 0.01
Sucrose	15	5.50 ± 0.01	5.55 ± 0.00
L. curvatus 1.624	2	5.51 ± 0.01	5.54 ± 0.01
L. curvatus 1.624	15	5.51 ± 0.01	5.49 ± 0.01
L. sakei 1.411	2	5.48 ± 0.02	5.53 ± 0.01
L. sakei 1.411	15	5.48 ± 0.02	5.48 ± 0.00
L. plantarum 1.1478	2	5.56 ± 0.02	5.57 ± 0.02
L. plantarum 1.1478	15	5.56 ± 0.02	5.56 ± 0.01
L. plantarum 1.25	2	5.52 ± 0.05	5.54 ± 0.03
L. plantarum 1.25	15	5.52 ± 0.05	5.55 ± 0.02

[a] Numbers are means ± standard deviation from duplicates, each examined three times ($n = 6$).

formed when both *Lactobacillus* ssp. and sugars are present in the meat samples. Experiments were hence carried out with raw minced meat and model systems containing either sugar **or** one of the mentioned LAB strains. Again, all samples (except the minced raw meat samples) were injected with brine and stored for 24 h at either 2 °C or 15 °C. In all minced raw meat samples, the anaerobic cell counts ranged between 0 and $10^2 \frac{CFU}{g\ meat}$ while the aerobic cell counts ranged between 10^2 and $10^3 \frac{CFU}{g\ meat}$ (*here:* be $3.40 \cdot 10^3$ and $4.00 \cdot 10^2 \frac{CFU}{g\ meat}$, respectively) indicating a good raw material quality (Feiner, 2006). The pH values of all minced raw meat samples analyzed ranged between 5.39 and 5.65. Table 1 summarizes the pH values of all model systems containing either one of the respective cultures or sugar (proof of concept). The viable cell counts of all strains examined (data not shown) remained approx. at the same level during 24 h of incubation ($\sim 10^6 \frac{CFU}{g\ meat}$). The pH values of the cooked ham model systems were 5.52 ± 0.4 and were hence in the range of EPS production (De Vuyst, Vanderveken, Van De Ven, & Degeest, 1998). The results demonstrate that the investigated strains are able to tolerate, but not to grow or produce acid under the harsh environmental conditions (1.92% salt; 2 °C and 15 °C).

Results of the CLSM investigation of model systems containing either brine with sugar, *L. plantarum* 1.1478, or *L. sakei* 1.411 are exemplarily shown in Fig. 2(proof of concept). In all pictures taken directly after mincing and staining, only very few EPS could be detected regardless of whether sugar or *Lactobacillus* ssp. were present in the meat samples. Furthermore, the EPS content did not increase during storage independent of the temperature used (24 h; 2 °C or 15 °C). The very small amounts of EPS that could be detected in these samples (Fig. 2) may be attributed to the presence of the autochthonous meat microflora or to the added strains, which are able to produce small amounts of EPS to some extent without the addition of sugar. Frequently isolated bacteria species of meat products are *Pseudomonas* spp. and *Lactobacillus* spp. such as *L. sakei* 0–1 a commonly used meat starter culture (Hufner et al., 2007; Leroy, Verluyten, & De Vuyst, 2006; Lewus, Kaiser, & Montville, 1991; Oubois, Beaumier, & Charbonneau, 1979). As already demonstrated, lactic acid bacteria are able to produce EPS in dairy and bakery products and may thus be also be able to produce EPS in meat products (Bouzar et al., 1997; Jakob, Steger, & Vogel, 2012; Kives, Orgaz, & SanJosé, 2006; Rühmkorf et al., 2012). However, the results gained clearly demonstrate that the strains examined (*L. plantarum* 1.1478, or *L. sakei* 1.411) are not able to produce EPS without the addition of sugar. Moreover, it could be proven that qualitative EPS detection using CLSM is an appropriate method to determine *in-situ* produced EPS in meat samples.

3.2. Evaluation of cooked ham model systems

The growth kinetics of *L. plantarum* 1.1478 and 1.25, as well as of *L. sakei* 1.411 and *L. curvatus* 1.624, in meat over a storage time of 48 h at 2° and 15 °C are illustrated in Fig. 3. At 2 °C (**3A**) the LAB strains showed no increase of viable cell counts ($\sim 10^6 \frac{CFU}{g\ meat}$) whereas, at 15 °C (**3B**), the viable cell counts of the HoPS-producing strains *L. curvatus* 1.624 and *L. sakei* 1.411 increased to $\sim 10^8 \frac{CFU}{g\ meat}$. This could be due to the different paths of EPS production, which are less energy-intensive for HoPS-producing strains that are able to synthesize huge amounts of glucans and fructans out of sucrose (Rühmkorf, Jungkunz, et al., 2012; Rühmkorf, Rübsam, et al., 2012), whereas HePS-producing bacteria synthesize their EPS out of different substrates in a complex and energy-intensive biosynthesis which is related to the cell wall biosynthesis (Sutherland, 2001).

The stagnating growth of the LAB strains at 2 °C could be a positive sign for the formation of EPS, since it is a stress-induced process (Czaczyk & Myszka, 2007; Wingender et al., 2012). A study performed by van den Berg et al. (1995) showed that the production of EPS in a semi-defined medium increased with decreasing temperature (from 30 °C to 20 °C) and, simultaneously, the growth rate in the exponential phase decreased significantly, from 0.22 g/L/h to 0.16 g/L/h. Similar results have been reported for *Lactococcus lactis* and *Lactobacillus casei* (Cerning, Bouillanne, Landon, & Desmazeaud, 1992; Kojic et al., 1992) as well as for *L. sakei* 0–1 (Degeest, Janssens, & De Vuyst, 2001). In contrast to that, Prasanna, Grandison, and Charalampopoulos (2012) found that *Bifidobacterium longum* ssp. *infantis* CCUG 52486 and *Bifidobacterium infantis* NCIMB 702205 produced the highest amount of EPS at the optimal growth temperature (37 °C), whereas no EPS production could be detected at 25 °C. Similar results were reported for *Streptococcus thermophilus* and *Bacillus licheniformis* KS-17 (Li et al., 2016; Song, Jeong, & Baik, 2013).

Besides the temperature hurdle, the high salt content in meat products such as cooked ham is another stress factor that impacts microbial growth and EPS formation. Quesada, Béjar, and Calvo (1993) investigated the impact of salt content on the production of EPS by the halophilic Eubacterium *Volcaniella eurihalina*. The authors reported that the amount of EPS increased from 0.4 g/L to 3 g/L at a concentration of 0% wt/vol and 10% wt/vol salt in malt yeast (MY) medium, respectively. However, higher salt contents did not further increase or decrease the EPS production.

The typical taste and flavor of meat is achieved between the pH values of 5.4 to 5.8, and a pH value of around 5.6 to 6.0 is necessary for a good water binding in cooked ham (Heinz & Hautzinger, 2009). The pH values of the cooked ham model samples were around 5.50 and remained at the same level during storage, except for both the HoPS-producing strains, where a slight but significant decrease ($p < .05$) in the pH values could be measured at the storage temperature of 15 °C (decrease of 0.07–0.1). The optimal pH value range for EPS formation was determined for four different *Streptococcus thermophilus* strains to be between 5 and 7 (De Vuyst et al., 1998) and 5.5 for *Streptococcus thermophilus* 1275 (Zisu & Shah, 2003). Based on these results, it can be assumed that the pH values of the minced raw meat used and the cooked ham model systems were supportive with respect to EPS-formation in the experiments presented. Since tumbling is usually performed at temperatures ≤ 2 °C, the slight pH drop determined in samples that were stored at 15 °C does not negatively impact the potential of all the strains examined to be used for prospective cooked ham production.

Figs. 4 and 5 exemplarily show CLSM images of cooked ham model systems stored over a period of 48 h at 2 °C, containing either *L. plantarum* 1.1478 and dextrose, or *L. sakei* 1.411 and sucrose. As explained in the previous chapter, the raw material already contained very few EPS (Figs. 4A and 5A). The images after 10 and 24 h of storage (simulating tumbling and resting time) are important for the application

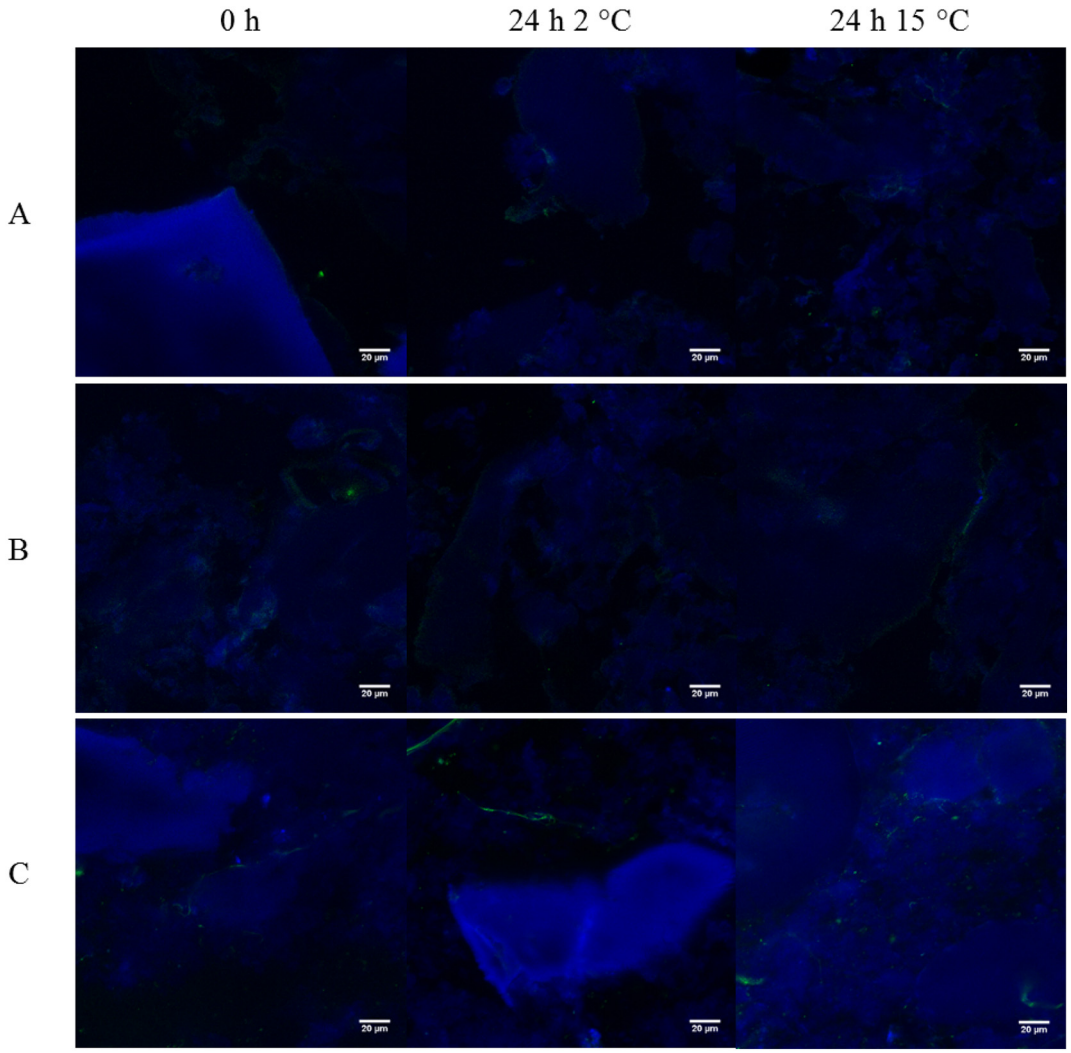

Fig. 2. Proof of Concept: Cooked ham model systems containing 1.92% nitrite curing salt, 0.03% ascorbate and either sucrose (sample A), *L. plantarum* 1.1478 (sample B), or *L. sakei* 1.411 (sample C). Cooked ham model systems were stored for 0 h, 24 h at 2 °C, or 24 h at 15 °C. EPS are stained green (Concanavalin A) and proteins are stained blue (Calcofluor White Stain); $n = 5$ pictures. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

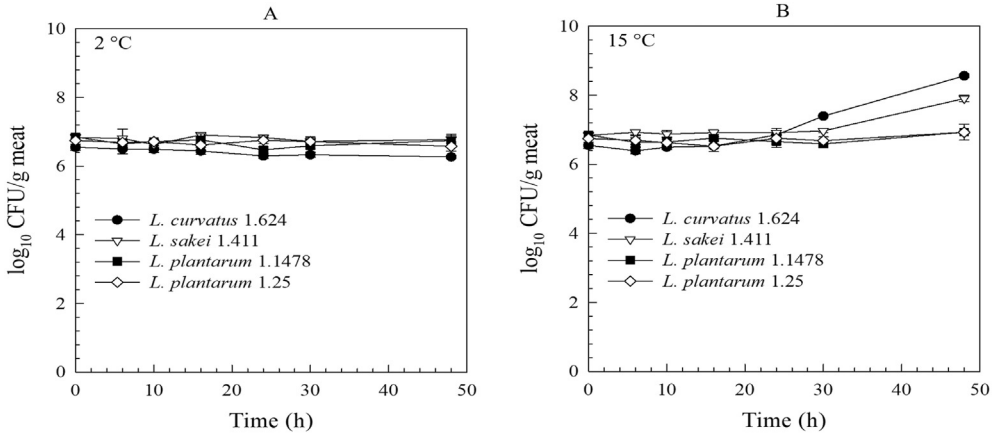

Fig. 3. Growth kinetics of *L. curvatus* 1.624, *L. plantarum* 1.1478, *L. plantarum* 1.25, and *L. sakei* 1.411 stored at 2 °C (3A) and 15 °C (3B) in a cooked ham model system containing 1.92% nitrite curing salt, 0.167% dextrose, and 0.03% ascorbate. Error bars are standard deviations from two independent replicates, each examined in duplicate ($n = 4$).

of EPS-forming bacteria in cooked ham. The amount of *in-situ*-produced EPS increased from 0 to 10 h to 24 h of storage. In both cases, no further increase in EPS formation could be observed after 48 h of incubation. Hassan et al. (2002) investigated the distribution of EPS in complex dairy matrices. There, proteins could be observed as distinct units, whereas EPS were present in the protein network pores. In the cooked ham model systems, the EPS detected (green) were located on the outer edges of the protein surfaces (blue) and no EPS could be found in the spaces between them (Figs. 4 and 5). Tables 2 and 3 demonstrate the image analysis of all CLMS images taken from the different cooked ham

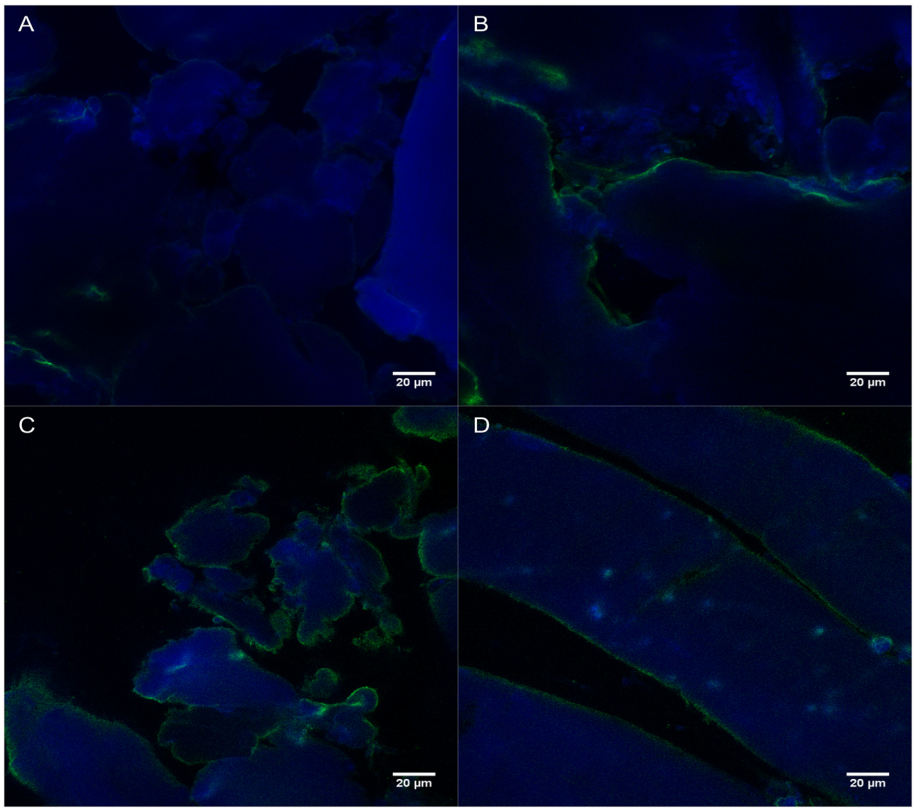

Fig. 4. Cooked ham model system containing homopolysaccharide-forming *L. sakei* 1.411, 1.92% nitrite curing salt, 0.167% sucrose, and 0.03% ascorbate. Sample A is the model system after production (0 h), whereas sample B, C, and D were stored for 10 h, 24 h, and 48 h at 2 °C, respectively. EPS are stained green (Concanavalin A) and proteins are stained blue (Calcofluor White Stain); n = 5 pictures. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

model samples that varied in respect of the bacterial strain and corresponding sugar used. Table 2 shows the results for samples containing HoPS-producing strains and sucrose. The amount of EPS detected, expressed as a green area (%), increased within the 10 h and 24 h *($p < .05$)* storage. As previously mentioned, it was also determined whether *L. curvatus* 1.624 and *L. sakei* 1.411 were also able to produce HePS if dextrose instead of sucrose is present in the meat samples (plausibility control). The plausibility control with dextrose showed

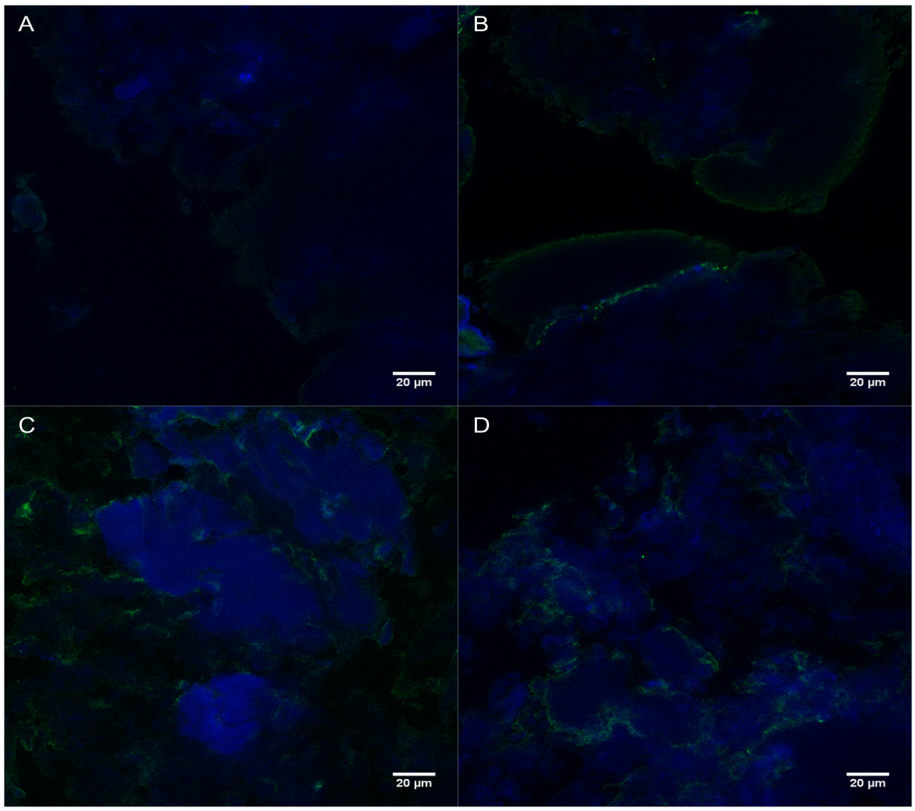

Fig. 5. Cooked ham model system containing heteropolysaccharide-forming *L. plantarum* 1.1478, 1.92% nitrite curing salt, 0.167% dextrose, and 0.03% ascorbate. Sample A is the model system after production (0 h), whereas sample B, C, and D were stored for 10 h, 24 h, and 48 h at 2 °C, respectively. EPS are stained green (Concanavalin A) and proteins are stained blue (Calcofluor White Stain); n = 5 pictures. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Results of the image analysis of CLSM pictures of cooked ham model systems containing the homopolysaccharide-producing strain *L. curvatus* 1.624 or *L. sakei* 1.411 (initial concentration ~10^6 CFU/g meat) and either brine with 0.5% sucrose or dextrose (plausibility control). Samples were stored at 2 °C and 15 °C over a period of 48 h.

Sample	Temperature (°C)	Storage time (h)	Mean green area* (%)	Standard deviation area (%)
L. curvatus 1.624 (sucrose)	2 °C	0	0.72[a]	0.66
		10	4.40[ab]	5.22
		24	3.32[ab]	3.10
		48	8.12[b]	3.17
L. curvatus 1.624 (sucrose)	15 °C	0	0.72[a]	0.66
		10	1.39[ab]	1.23
		24	8.41[c]	2.95
		48	3.87[b]	1.64
L. curvatus 1.624 (dextrose)	2 °C	0	1.63[a]	2.19
		10	2.08[a]	2.01
		24	1.82[a]	1.93
		48	3.75[a]	2.07
L. curvatus 1.624 (dextrose)	15 °C	0	0.71[a]	0.34
		10	1.41[ab]	1.04
		24	2.75[b]	1.91
		48	4.91[c]	1.63
L. sakei 1.411 (sucrose)	2 °C	0	1.33[a]	2.20
		10	4.94[ab]	2.03
		24	7.36[b]	5.34
		48	8.18[c]	3.23
L. sakei 1.411 (sucrose)	15 °C	0	2.61[a]	0.53
		10	4.23[ab]	5.52
		24	6.36[b]	3.02
		48	5.33[b]	2.12
L. sakei 1.411 (dextrose)	2 °C	0	1.33[a]	0.53
		10	4.46[b]	2.23
		24	2.43[ab]	1.40
		48	4.06[b]	2.40
L. sakei 1.411 (dextrose)	15 °C	0	1.33[a]	0.53
		10	3.41[a]	5.34
		24	2.62[a]	2.31
		48	3.54[a]	1.43

Values with different letters show significant differences ($p < .05$) within the strain-specific column (0 h – 48 h).
* n = 5 pictures.

Table 3
Results of the image analysis of CLSM pictures of cooked ham model systems containing the heteropolysaccharide-producing strain *L. plantarum* 1.1478 or 1.25 (initial concentration ~10^6 CFU/g meat) and 0.5% dextrose. Samples were stored at 2 °C and 15 °C over a period of 48 h.

Sample	Temperature (°C)	Storage time (h)	Mean green area* (%)	Standard deviation area (%)
L. plantarum 1.1478	2 °C	0	0.44[a]	0.29
		10	3.35[ab]	1.96
		24	12.08[c]	5.84
		48	5.11[b]	2.51
L. plantarum 1.1478	15 °C	0	0.44[a]	0.29
		10	5.07[ab]	3.51
		24	8.63[b]	3.75
		48	8.10[b]	5.23
L. plantarum 1.25	2 °C	0	2.64[a]	2.33
		10	7.81[ab]	3.15
		24	11.03[b]	5.13
		48	9.73[b]	5.70
L. plantarum 1.25	15 °C	0	4.04[a]	2.96
		10	13.09[b]	7.42
		24	9.91[ab]	8.00
		48	12.29[ab]	5.06

Values with different letters show significant differences ($p < .05$) within the strain-specific column (0 h – 48 h).
* n = 5 pictures.

lower increases in EPS production over time compared to samples containing sucrose. This result indicates that the examined HoPS-forming strains may also be able to produce HePS to some extent. However, the performance of *L. curvatus* 1.624 and *L. sakei* 1.411 in the presence of sucrose was found to be much better. Similar findings were made by van den Berg et al. (1995) and Van der Meulen et al. (2007) and could be an interesting topic for further research. Table 3 summarizes the results of the image analysis of samples containing dextrose and the HePS-producing strains *L. plantarum* 1.1478 and 1.25, which were found to produce significant *($p < .05$)* amounts of EPS during 24 h of storage. The calculation of the areas and mean areas (Tables 2 and 3) based on the results illustrated in Figs. 4 and 5 indicates that the initially performed qualitative interpretation of the pictures correlates well with the related calculations and, hence, the comparison between the different samples and thus between the strains became more expressive. In summary the results showed that the HoPS- and HePS-forming strains were able to produce EPS in significant ($p < .05$) amounts during 24 h of incubation at 2 °C (important parameter for cooked ham production) and 15 °C. Moreover, the HePS-forming strains were found to produce significantly ($p < .05$) higher amounts of EPS in this period of time than the HoPS-producing strains.

Decreases in the values for the (mean) green surface area could be observed after 48 h of storage, which could be attributed to an enzymatic degradation (Pham, Dupont, Roy, Lapointe, & Cerning, 2000).

Isolated EPS are already known for their ability to improve product characteristics and are used in the food industry. For instance, 1% *w/w* *in-situ*-produced, isolated and separately applied dextran improved the moisture content, baking loss, and crumb firmness of breads made of buckwheat and rice flour (Rühmkorf, Rübsam, et al., 2012). In another study performed by van den Berg et al. (1995), isolated EPS from *L. sakei* 0–1 were analyzed regarding their exopolysaccharide composition and viscosifying properties. The HePS were composed of glucose and rhamnose and were found to have better viscosifying properties than xanthan gum at a concentration of 1%. Nevertheless, the application of *in-situ* EPS-producing strains instead of isolated EPS was also already found to have great potential in improving product characteristics. For instance, *L. curvatus* 1.624 was found to be able to produce EPS in bread dough which could be correlated to an overall improved quality in gluten-free breads (Rühmkorf, Jungkunz, et al., 2012). Moreover, in contrast to isolated EPS, *in-situ*-produced EPS do not have to be labeled.

4. Conclusion

Taking the results of the present study into account, the examined LAB strains have a great potential to be used in cooked ham production. Both HoPS-producing *L. sakei* 1.411, *L. curvatus* 1.624 and HePS-producing strains *L. plantarum* 1.1478 and 1.25 were found to not only produce EPS at 15 °C but also at 2 °C within the first 10 to 24 h of storage, which is essential for the tumbling process step during cooked ham production. Moreover, all strains were able to tolerate the high salt content (1.92% NCS). No increase in viable cell counts could be determined at 2 °C, and the pH values were found to be in the perfect range for a good cooked ham quality and for optimal EPS-production, which may cause an improved water-holding capacity, probably leading to juicier products.

The CLSM analysis proved to be a suitable qualitative method to detect EPS in meat matrices and became even more expressive through the calculation and comparison of percentage changes of areas, which could be directly correlated with changes in the EPS amounts over time. Nevertheless, a quantitative EPS analysis should additionally be performed during cooked ham production in order to better correlate the effect of water binding to the amount of present EPS. Further interesting research questions to focus on are, for instance, the examination of structural differences of products that have been produced with isolated EPS in comparison to those containing *in-situ* EPS-producing *Lactobacillus* ssp., and the resulting influence on product properties.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2018.10.058.

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