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In situ production and characterization of cloud forming dextrans in fruit-juices

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ABSTRACT

Turbidity in beverages is typically achieved by addition of emulsion based cloud systems. Their intrinsic instability necessitates the widespread use of technological measures and use of food additives to prevent emulsion decay. In this work, we explored the possibility to establish a new generation of natural, stable clouding systems based on bacterial dextrans. *Lactobacillus hordei* TMW 1.1907 originating from water kefir was used to produce dextrans in sucrose supplemented apple or grape juices. By varying the fermentation conditions, two distinct types of dextran molecules could be produced at yields ranging from 2.5 to 8.5 g/L. The dextran-containing fermentates showed an unchanged turbidity after pasteurization at acidic pH and subsequent storage for three months. No sedimentation of particles occurred upon storage. Neutralization of the acidic fruit juices to pH 7 prior to fermentation significantly increased the dextran yields. The molecular weight, rms radii and turbidity of dextrans produced at 20 °C were higher than those produced at 30 °C. Characterization of the isolated dextrans by asymmetric flow field-flow fractionation coupled to multi-angle laser light scattering revealed a random-coil like structure and rms radii ranging from 66.0 to 87.4 nm. The averaged molar masses of the cloud forming dextrans were in the approximate range of 103.1 to 141.6 MDa. In conclusion, our results demonstrate the possibility to ferment fruit juices for *in situ* production of dextrans exhibiting novel techno-functional properties beyond gelling and thickening.

1. Introduction

Turbidity in fruit-juice derived beverages is typically achieved by addition of artificial cloud systems (Taherian et al., 2007). These contain dispersed oil in water emulsions with oil droplet sizes ranging from 0.2 to 5 µm (Dickinson, 1994; Linke and Drusch, 2016). The international demand for these cloud systems is expected to grow in the future (Transparency Market Research, 2017).

Oil-in-water-emulsions are thermodynamically unstable. One major reason is that the density difference between oil and water usually results in a creaming of the oil phase (Tan, 1998). This effect is based on the fact that the settling velocity of a droplet in a liquid accelerates for higher density differences, larger droplet sizes and lower continuous phase viscosities (McClements, 2005; Stokes, 1845). The same holds true for the creaming of these oil-in-water emulsions, where oil droplets can be regarded as particles (McClements, 2005).

Stabilizers retard the instability in food emulsions like beverages (Cao et al., 1990). In foods, high-molecular weight polysaccharides like xanthan, carrageenan and carboxmethylcellulose are used as stabilizers (Cao et al., 1990). Of these commonly used polysaccharides, the exopolysaccharide (EPS) xanthan is of bacterial origin and serves as stabilizer for cloud-forming oil-in-water emulsions (Mirhosseini et al., 2008).

Many food-grade lactic acid bacteria (LAB) also synthesize EPS (Monsan et al., 2001; Moonmangmee et al., 2002), which can be produced *in situ* in foods by starter cultures without the need of EPS isolation and labeling on food packages in contrast to commercially available hydrocolloids (Caggianiello et al., 2016). The positive effects of these molecules are traditionally exploited in the production of dairy products such as e.g. yoghurt and cheeses (Cerning, 1990). In the case of cheese production, the production of heteropolysaccharides is partly responsible for enhanced rheological properties of the food products (Vuyst and Degeest, 1999). Apart from heteropolysaccharides, some LAB produce homopolysaccharides like dextrans, whose backbones are

Abbreviations: EPS, Exopolysaccharides; LAB, Lactic acid bacteria; *Lb*, *Lactobacillus*; AF4, asymmetric flow field-flow fractionation; MALLS, multi-angle laser light scattering; A7, 25% apple juice with 40 g/L sucrose and pH adjusted to 7; G7, 25% grape juice with 40 g/L sucrose and pH adjusted to 7; An, 25% apple juice with 40 g/L sucrose; Gn, 25% grape juice with 40 g/L sucrose

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composed of α-(1 → 6)-linked glucose moieties (Monsan et al., 2001). Dextrans are synthesized by extracellular enzymes, namely dextransucrases (EC 2.1.4.5) that use the energy of the glycosidic bond in sucrose for transglycosylation reactions (Cerning, 1990). Many lactic acid bacteria that are commonly found in water kefir, including *Lb. hordei* and *Lb. hilgardii*, produce homopolysaccharides like dextrans (Fels et al., 2018; Gulitz et al., 2011; Laureys, 2017). The use of dextrans, particularly from *Leuconostoc mesenteroides*, ranges from analytical (*e.g.* chromatography columns, molecular weight standards) (Monsan et al., 2001) and baking applications (e. g. panettone) (Decock and Cappelle, 2005) to medical uses like blood plasma substitutes (Naessens et al., 2005).

Some of our previous works revealed that high molecular weight fructans (levans) from diverse acetic acid bacteria and dextrans from *Lactobacillus sakei* increase the turbidity of aqueous solutions due to their spherical character at high molecular weight ($> 10^8$ g/mol), which results in intense light scattering (Jakob et al., 2013, Ua-Arak et al., 2017, Prechtl et al., 2018). Hence, such polysaccharides could be used as cloud or turbidity-forming agents in beverages, provided that they do not thicken liquids at the used concentrations and that no degradation or sedimentation effects occur upon storage. In practice, using polysaccharides as cloud forming agents would mean less processing steps in the manufacturing of turbid lemonades, giving the opportunity to reduce costs and to limit the amount of necessary additives. This omission of additives, by natural ingredients or *in situ* production is again in the interest of consumers, which are increasingly looking for more natural foods produced with less additives (Asioli et al., 2017).

The goal of this study was to investigate the possibility to produce cloud forming dextrans *in situ* in fruit juices by LAB isolated from the traditional beverage water kefir, which is fermented by diverse dextran producing LAB (Fels et al., 2018; Xu et al., 2018). Accordingly, we wanted to find suitable conditions for dextran production by the selected LAB in fruit juices and correlate the macromolecular structures of the produced dextran molecules with their cloud forming properties.

2. Material and methods

2.1. Cultivation of Lactobacillus strains

Lb. hordei TMW 1.1822, *Lb. hordei* TMW 1.1907, *Lb. hilgardii* TMW 1.828 and *Lb. hilgardii* TMW 1.2196 have been isolated from water kefir (Gulitz et al., 2011). Both *Lb. hordei* strains produce dextran from sucrose, while the used *Lb. hilgardii* strains produce so far non characterized glucans from sucrose (Gulitz, 2013; Xu et al., 2018). The strains were generally cultivated in a modified MRS medium according to Gulitz et al. (2011). Agar plates were incubated under anaerobic conditions (Anaerogen, Oxoid) and liquid cultures were prepared in a 15 mL screw-top tube filled with 15 mL of medium and an atmospheric headspace. Pre-cultures were prepared by static, anaerobic incubation at 30 °C in liquid mMRS medium for 48 h. Cells grown to the stationary phase were used as starter culture for fruit juice fermentations. Viable cell counts were determined by serial dilutions of the fermentates with 0.9% NaCl solution and subsequent plating on mMRS agar plates using glass beads as duplicate. Incubation took place aerobically at 30 °C for 48 h.

2.2. Determination of viscosities

MRS medium with 40 g/L sucrose as sole carbon source (sucMRS) were inoculated from pre-cultures to an OD_{600} of 0.1. After fermentation for 48 h at 20 °C, flow times were determined in triplicate using a flow-cup according to DIN 53211 and a stop watch.

2.3. Fermentation of selected fruit juices

Main fermentations were carried out in fruit juice based media, which consisted of clear commercial apple or grape juice (Wolfra, Erding, Germany) that was diluted 1:4 and supplemented with 40 g/L sucrose as fermentation substrate. Apple (A) and grape (G) juices were used at their native pH (An and Gn) as well as with a pH adjusted to pH 7 (A7 and G7). Media were sterile filtered using a CytoOne 0.2 µm bottle top filter (Starlab international GmbH, Hamburg, Germany) after preparation. Fermentation media were inoculated as single cultures to an optical density at 590 nm of 0.1 from pre-cultures of the respective strains. Small-scale fermentations were carried out in 15 mL screw-top tubes containing 15 mL of medium. Large-scale fermentation was carried out in 2 L laboratory-grade screw-top glass bottles. After fermentation, the fermentates were divided: 0.5 L were directly used for stability testing. These 0.5 L were cooled to 4 °C and centrifuged at 10,000 × *g*, 10 min at 4 °C in order to remove cells. The supernatant was subjected to this treatment again in order to remove all cells. The remaining 1.5 L were distributed to 0.5 L centrifuge beakers, which were subsequently cooled to 4 °C using an ice bath. After reaching 4 °C, cells were likewise removed. This supernatant was used for EPS purification as described below.

2.4. Dextran purification

EPS-containing solutions were precipitated using 2 volumes of ethanol chilled to −20 °C. After incubation at 4 °C over night, the precipitate was harvested using centrifugation (10 min, 10,000 × *g*, 4 °C) and resuspended in water. This resuspended EPS was filled into dialysis tubes (MWCO 3500 Da) and dialysis against dH2O was carried out at 4 °C for 48 h. After dialysis, samples were freeze dried at −20 °C.

2.5. Determination of dextran yields

Dextran yields from fermentations were determined gravimetrically in triplicates for each condition. For this purpose, fermentations were carried out according to the standard conditions. After 48 h, the fermentates were cooled to 4 °C and cells were removed by centrifugation at 10,000 × *g*, 10 min at 4 °C. Dextrans were purified according to the protocol given above and the amount of dextran was gravimetrically determined after freeze drying.

2.6. Heat treatment of raw fermentates and purified EPS solutions

Fermentates and EPS solutions were heat treated in order to achieve microbial stability. For this purpose, 500 mL of each sample was transferred to a 500 mL laboratory-grade screw-top glass bottle. 0.5 L samples were heat treated in a VX 150 autoclave (Systec, Linden, Germany) with a water filled reference bottle for temperature reference. The autoclave was programmed to heat the bottles to 85 °C for 3 min, a heat treatment described for grape juice (Zhao, 2012). After cooling to 80 °C, the bottles were removed from the autoclave and cooled to room temperature. Laboratory scale heat treatment was conducted using a heating block with the same treatment regime.

2.7. Long term storage of dextran-containing solutions

Raw fermentates and isolated dextrans were stored over 3 months. Isolated dextran was resuspended at the same concentration as was present in the respective fermentates in a single 2 L fermentation (6.54 g/L for A7, 20 °C and 9.90 g/L for A7, 30 °C) in phosphate-citrate buffer prepared according to McIlvaine (1921) at pH 3. After heat treatment, dextran containing solutions were stored statically in the dark at room temperature. For weekly sampling, screw-top bottles were removed with minimal disturbance. Samples were taken sterilely from the top 2 cm of the bottle using a pipette. Optical density at 400 nm was

determined weekly using a spectrophotometer.

2.8. Quantification of sugars and acids

Sucrose, fructose, glucose, acetic acid, lactic acid, malic acid, citrate, lactate, acetate, ethanol, mannitol and succinate were determined by high pressure liquid chromatography (HPLC). The samples were filtered with 0.20 µm syringe filters (Phenomenex, USA) before HPLC analysis. For separation of sugars, a Rezex RPM column (Phenomenex, USA) and for separation of acids, a Rezex ROA column (Phenomenex, USA) was used at a flow rate of 0.6 mL/min and ddH_2O or 0,025 M H_2SO_4 as eluents, respectively.

2.9. Determination of extinction coefficients of the isolated dextrans

In order to determine the extinction coefficients of the isolated dextrans, 5 solutions of 0.5, 1, 2, 3 and 4 mg/mL were prepared in dH_2O, respectively. The extinction of these solutions were measured in triplicates in 96 well microtiter plates using a plate reader and the resulting data was used for the calculation of the extinction coefficient by calculating the resulting slope.

2.10. Structural analysis of produced dextrans by asymmetric flow field-flow fractionation (AF4) coupled to multi-angle laser light scattering (MALLS)

Dextrans produced by *Lb. hordei* were separated and analyzed by AF4 (Wyatt Technology, Germany) coupled to MALLS (Dawn Heleos II, Wyatt Technology, Germany) and UV detection (Dionex Ultimate 3000, Thermo Fisher Scientific, USA). The dextrans were isolated and dissolved in 50 mM $NaNO_3$ to a concentration of 1 mg/mL. 100 µL were subsequently injected into the separation channel. The separation method used was modified from Ua-Arak et al. (2017), using 0.2 mL/min injection flow and 1 mL/min elution flow. The gradient cross flow rate was set from 3 mL/min to 0.1 mL/min within 10 min and kept at 0.1 mL/min for 30 min. The separations were performed on 10 kDa regenerated cellulose membranes (Superon GmbH, Germany) with 50 mM $NaNO_3$ (aq.) as eluent solution. Extinction coefficients were obtained experimentally for each dextran sample. The dn/dc value for dextran was set to 0,1423 mL/g according to (Yuryev et al., 2007). The recorded data were analyzed by the ASTRA 6.1 software (Wyatt Technology, Germany).

2.11. Genome sequencing and analysis

Genomic DNA from L. *hordei* TMW 1.1907 was isolated using the E.Z.N.A® Bacterial DNAKit (Omega Bio-Tek Inc., Norcross, GA, USA) following the manual. Genome sequencing of strain TMW 1.1907 took place with a MiSeq sequencing platform (Illumina, Inc., San Diego, USCA) using a PCR-free library preparation. Preparation, processing and assembly was done with SPAdes V3.9.0 (Bankevich et al., 2012) according to the method described by (Huptas et al., 2016). Annotation was conducted using the NCBI Procaryotic Genome Annotation Pipeline (Haft et al., 2018; Tatusova et al., 2016).

3. Results

3.1. Preliminary strain selection

In a first step, diverse dextran producing LAB strains (*Lb. hilgardii*, *Lb. hordei*) isolated from water kefir were screened for their capability to produce non-thickening dextrans in sucrose supplemented nutrient media according to 2.1–2-3. The flow times for sucMRS fermented with *Lb. hilgardii* TMW 1.828 and 1.2196 were 219 ± 20 s and 132 ± 32 s, respectively. The flow times for sucMRS fermented with *Lb. hordei* TMW 1.1822 and 1.1907 were 10 ± 0 s in both cases, which was comparable to the flow time of unfermented nutrient media (10 ± 0 s). Hence, the dextrans of both *Lb. hordei* strains did not increase the viscosity of fermentation broths in contrast to those produced by *Lb. hilgardii*. However, subsequent tests revealed that *Lb. hordei* TMW 1.1822 grew poorly in fruit juice based media. Therefore, *Lb. hordei* TMW 1.1907 was selected for the upcoming experiments.

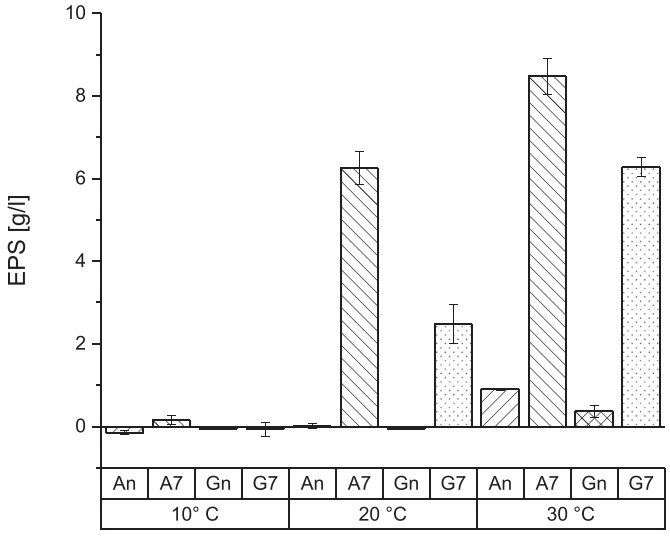

Fig. 1. Dextran yield in g/L from the fermentation of 4 fruit juice based media fermented at 10, 20 and 30 °C. Fermentations were carried out in 15 mL scale for 48 h.

3.2. Fermentation of fruit juice based media

To investigate the suitability of *Lb. hordei* TMW 1.1907 to produce cloud forming dextrans in fruit juice based media, four different media were tested at 10, 20 and 30 °C. The dextran yields obtained from fermentations after 48 h are shown in Fig. 1, cell counts are listed in Table 1. The highest dextran yields were obtained in pH adjusted media, while in non-pH adjusted media no dextran production at 10/20 °C occurred. Low amounts of dextran were produced at 30 °C in non pH-adjusted media. Hence, EPS production was more efficient, if the pH of the fruit juices had been preliminary adjusted to pH 7. However, despite the low dextran production, *Lb. hordei* TMW 1.1907 was able to grow in native juices (Table 1). The cell counts after 48 h not shown in Table 1 are: $1.5 \pm 0.5 \times 10^8$ cfu/ml (An at 20 °C) $1.2 \pm 0.6 \times 10^7$ cfu/mL (Gn at 20 °C), $1.7 \pm 0.6 \times 10^8$ cfu/mL (An at 30 °C) and $8.2 \pm 0.2 \times 10^7$ cfu/mL for native pH fruit juices. The fermentation pH values dropped over the course of 48 h from pH 7 to pH 4.4 and 4.7 (A7, G7 at 20 °C) and from pH 7 to pH 3.9 and 4.1 (A7, G7 at 30 °C). The concentration of sugars and acids in the (fermented) fruit juices were determined by high pressure liquid chromatography (Table 1). Ethanol production was not detected. Acetate was detected at low levels after fermentation, but was below the limit of quantification, while lactate concentrations increased over the fermentation time. The sucrose concentrations decreased during fermentations, while the overall sucrose consumption was higher in grape juice based fermentations compared to apple juice based fermentations. Fructose and malate concentrations increased or decreased upon fermentation, respectively.

3.3. Characterization of the macromolecular properties of in situ produced dextrans

AF4-MALLS-UV was used to characterize the *in situ* produced dextrans produced by *Lb. hordei* TMW 1.1907 with regard to molecular size. In order to determine the molecular structure of the produced

Table 1
Cell counts and concentration of selected metabolites before and after fermentation as determined by plate count and HPLC analysis.

	Cell count [cfu/mL]	Sucrose [mmol/L]	Glucose [mmol/L]	Fructose [mmol/L]	Malate [mmol/L]	Lactate [mmol/L]
A7 0h	6.2 ± 0.5 × 10^6	111.2 ± 3.2	35.3 ± 0.6	63.2 ± 1.7	272.1 ± 5.5	n.d.
A7 48h 20 °C	1.8 ± 0.8 × 10^6	3.3 ± 0.7	18.3 ± 0.5	158.9 ± 9.1	33.6 ± 0.4	32.3 ± 0.2
A7 48h 30 °C	1.4 ± 0.3 × 10^8	6.3 ± 0.3	18.5 ± 0.9	169.7 ± 10	125 ± 69.9	34.7 ± 9
G7 0h	6.2 ± 0.5 × 10^6	124.6 ± 0.6	122.9 ± 0.6	133.4 ± 0.7	372.8 ± 11.3	n.d.
G7 48h 20 °C	1.9 ± 1.0 × 10^8	11.8 ± 0.1	93.6 ± 2.2	221.2 ± 4.3	151.1 ± 4.2	39.1 ± 1.2
G7 48h 30 °C	2.0 ± 0.2 × 10^8	13.7 ± 0.1	90.9 ± 1.2	206.5 ± 2.6	144.1 ± 3.3	46.6 ± 1.5

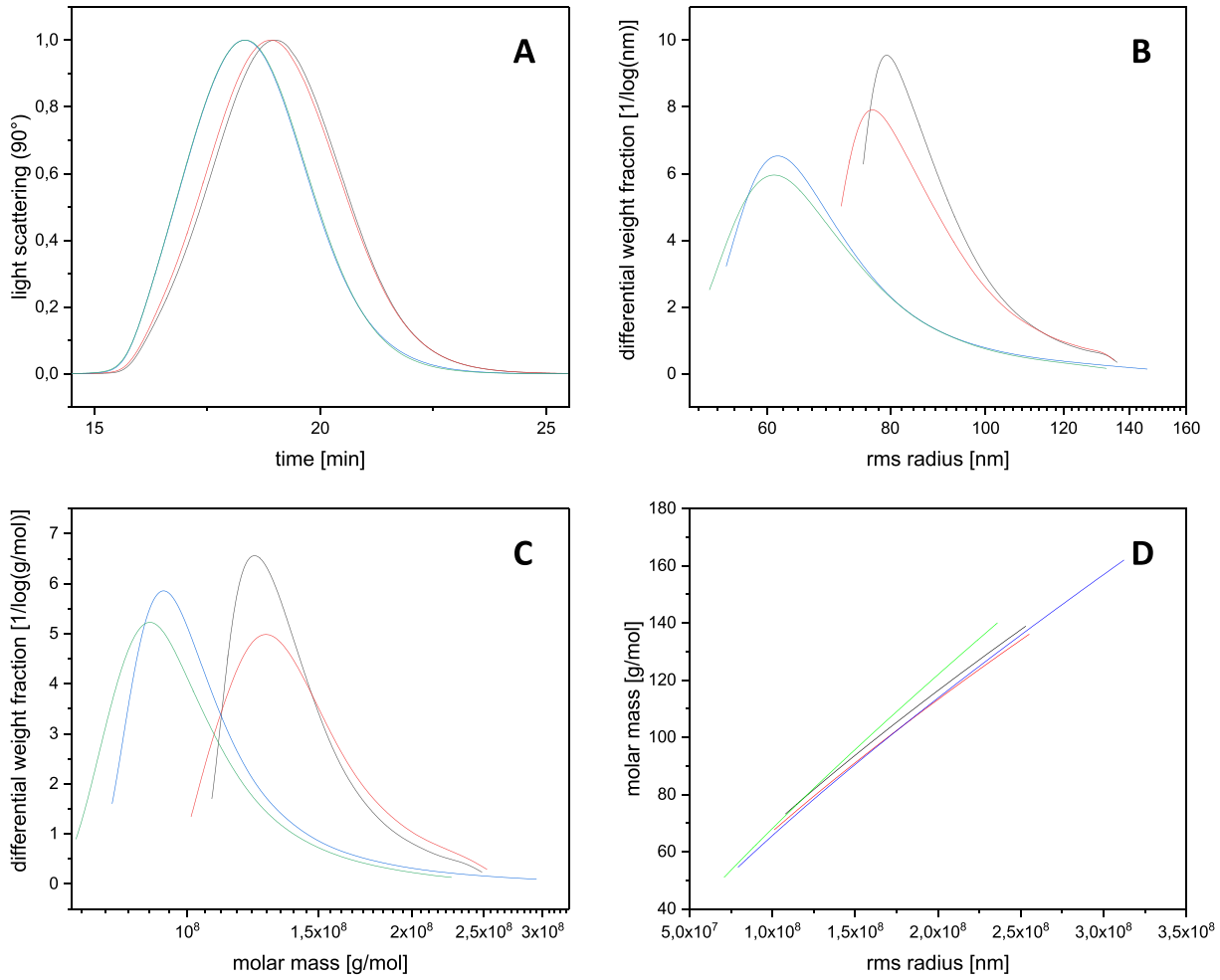

Fig. 2. Separation profiles and sizes of *in situ* produced dextrans. AF4-MALLS chromatograms (A), differential weight distributions of rms radii (B) and molar masses (C), and conformation plots (D). Black: A7 20 °C, red: G7 20 °C, blue: A7 30 °C, green: G7 30 °C. The Berry model was used for calculation of molar masses and rms radii. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Key features of analyzed dextrans as obtained from AF4 MALLS analysis.

		r (avg, nm)	Mw (mDa)	Retention time (min)	Conformation plot slope	R^2 (Berry model)	R^2 (random coil model)	R^2 (rod model)
20 °C	A7	87.4	141.2882	18.965	0.75	0.9984	0.9956	0.9774
	G7	85.5	141.5691	18.864	0.76	0.9989	0.9975	0.9816
30 °C	A7	66.1	111.8581	18.241	0.79	0.999	0.9985	0.9973
	G7	66.0	103.0909	18.258	0.84	0.9992	0.9987	0.9976

dextrans, conformation plots were additionally generated using the ASTRA 6.1 software. The obtained chromatograms, rms-radius distributions, molecular weight distributions and conformation plots are depicted in Fig. 2. The weight averaged molecular weights and rms radii, the hydrodynamic coefficients ν_G (slopes) obtained from conformation plots (Fig. 2D) and the coefficients of determination (R^2) obtained using diverse evaluation models at the respective peak maxima (Fig. 2A) are summarized in Table 2. Dextrans produced at the same temperatures exhibit comparable molecular weights and rms radii independent of the used fruit juice, respectively (Fig. 2A–C, Table 2). According to shifted retention times (20 °C *vs.* 30 °C; Fig. 2A), dextrans produced at 20 °C were comparatively larger in size. Although the Berry

algorithm generally allowed the best fit (highest R^2) for all separated dextrans, the obtained fit for random coil is still similarly high (Table 2), suggesting the molecular structure to most closely resemble a random coil like form. Evaluation of the conformation plots further revealed, that all slopes obtained from the respective regression lines are similarly located in between the values for a typical random coil ($\nu_G \sim 0.5–0.6$) and rod ($\nu_G \sim 1$) molecule (Jakob et al., 2013; Nilsson, 2013), as shown in Table 2. The macromolecular structure of the *in situ* produced dextrans in aqueous solution might thus resemble a stretched random coil molecule, respectively, while smaller dextrans produced at 30 °C are less compact and more elongated (higher ν_G) in contrast to their larger counterparts produced at 20 °C. Accordingly, R^2 is still > 0.99, if the rod model is used for evaluation of the dextrans produced at 30 °C (Table 2).

3.4. Stability of the in situ produced dextrans towards hydrolysis upon heat-treatment

The stability of the produced glucans towards acidic hydrolysis upon heat treatment was assessed using AF4-MALLS. For this purpose, dextrans were dissolved in McIlvaine buffer (pH 3 or pH 7), heat treated and afterwards compared to the respective non-heat treated samples (controls). Heat treated dextrans dissolved at pH 3 showed a reduced molecular weight and rms radius in contrast to those dissolved at pH 7, as shown for dextrans produced at 30 °C in Fig. 3. However, this heat treated dextran fraction with reduced molecular weight still possessed sufficient turbidity-forming properties for both glucans produced at 30 °C and 20 °C, as is also evident in Fig. 4.

3.5. Stability of the dextran-based cloud systems towards hydrolysis during long-term storage

The stability of the dextrans towards spontaneous hydrolysis at room temperature was further determined by a long-term storage experiment. For this purpose, the fermented, dextran-containing fruit juice-based media as well as the isolated dextrans from these fermented fruit juices redissolved in McIlvaine buffer (pH 3) were stored over 3 months. As a measure of turbidity, the optical densities at 400 nm (OD_{400}) were recorded weekly. In the dextran-containing fruit juice fermentates (Fig. 4A), the OD_{400} decreased during the first 3 weeks of storage until a steady level was reached. This stable level of turbidity approximately corresponded to the level of turbidity achieved by dissolving the isolated dextrans from fruit juices in McIlvaine buffer (pH 3). The levels of turbidity of the isolated dextrans were stable over the course of the experiment after week 3. Moreover, no sedimentation of particles was observed upon storage.

The cloud forming properties of the *in situ* produced glucans after 3 months of storage are depicted in Fig. 4B. On the contrary, in unfermented fruit juices no clouding is observed (Fig. 4C).

4. Discussion

4.1. Strain selection

The used *Lb. hordei* and *Lb. hilgardii* strains of this study are part of the natural water kefir microbiota (Gulitz et al., 2011; Laureys, 2017). This underlines their suitability to ferment acidic, high sugar media like water kefir or fruit juices, which are otherwise poor in nitrogen sources. However, preliminary screenings revealed that dextrans produced by diverse water kefir born *Lb. hilgardii* strains exhibited thickening properties, which is an undesired feature of EPS being typically associated with spoilage of beverages (Fraunhofer et al., 2018; Werning et al., 2006). On the contrary, the dextrans produced by both tested *Lb. hordei* strains exhibited no thickening effects, but increased the turbidity of solutions implying their possible suitability as cloud forming agents. Growth of dextran producing water kefir LAB in fruit juices has not been investigated before, but was expected to be possible due to comparable fermentation conditions in regards to high sugar and low nitrogen contents. *Lb. hordei* TMW 1.1822 showed poor growth in fruit juices in contrast to *Lb. hordei* TMW 1.1907, which was subsequently selected for further experiments. Not each strain of L. *hordei* isolated from water kefir is hence able to grow in fruit juices implying the complex metabolic adaptations observed among diverse water kefir organisms (Bechtner et al., 2019; Xu et al., 2019; Xu et al., 2018).

4.2. Production of clouding dextrans in fruit-juice-based fermentation media

Lb. hordei TMW 1.1907 was isolated from water kefir previously and produces dextrans from sucrose (Xu et al., 2018). The obtained dextran yields in the tested apple and grape juices were generally lower than those reported in MRS supplemented with 8% (w/v) sucrose (Stadie, 2013), which could be due to the limited nutrient content in fruit juices. However, growth also occurred in non-pH adjusted fruit juices (Table 1), while no (20 °C) or solely low levels (30 °C) of dextran were produced in these dilute juices (Fig. 1). The dextransucrase was thus either not properly operating at 20 °C or had not been released at all at this temperature. Moreover, the environmental pH seems to be crucial

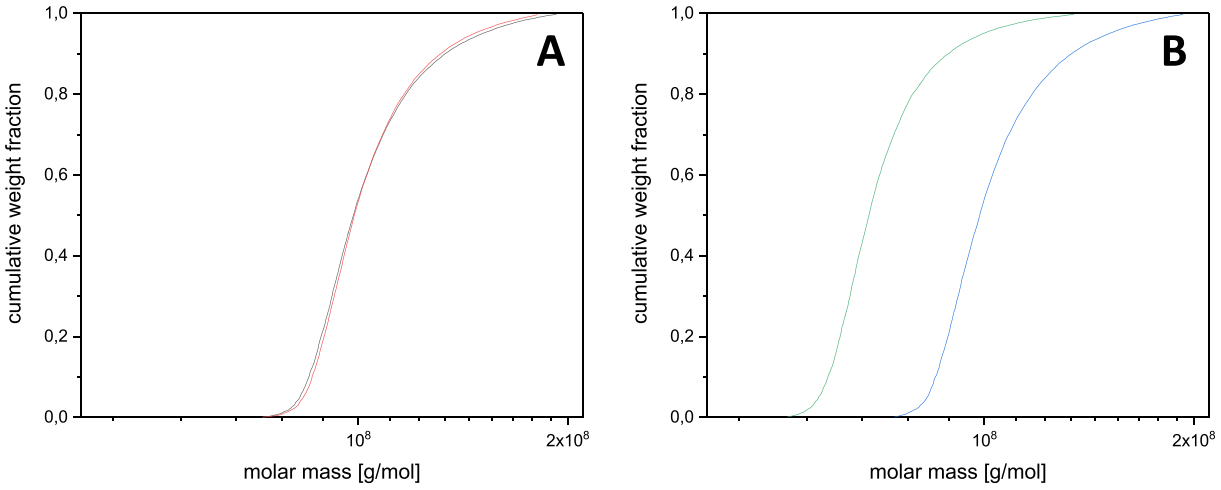

Fig. 3. Cumulative molar mass distributions of A7 30 °C dextran before and after heat treatment. Panel A shows data for dextran dissolved in pH 7 phosphate-citrate buffer before (black line) and after (red line) heat treatment. Panel B shows data for dextran dissolved in pH 3 phosphate buffer before (blue line) and after (green line) heat treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

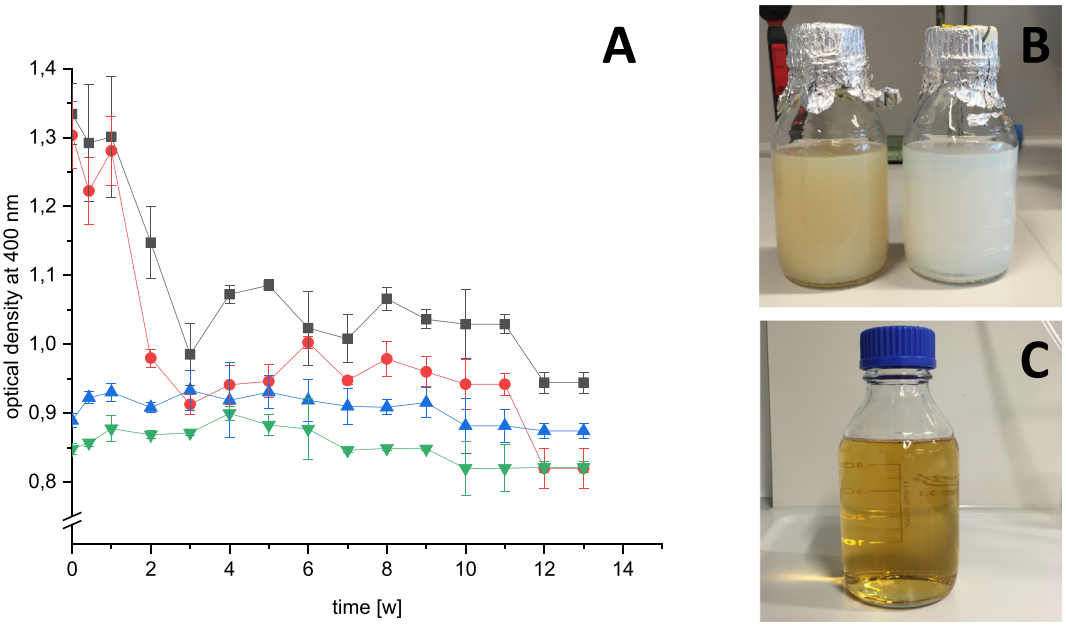

Fig. 4. Turbidity development of dextran-containing solutions upon storage at room temperature for 13 weeks. A: Recorded OD_{400} values of the different dextran solutions at different weeks (w) of storage; grey square: apple juice fermented at 20 °C (A7, cell-free), red square: apple juice fermented at 30 °C (A7, cell-free), blue triangle: redissolved dextran from apple juice (A7, 20 °C); green triangle: redissolved dextran from apple juice (A7, 30 °C). B: turbidity of dextran-containing, fermented apple juice (left; A7, 30 °C, 48 h, cell-free) and of the corresponding isolated dextran redissolved at the same concentration (6.54 g/L for A7, 20 °C and 9.90 g/L for A7, 30 °C) in McIlvaine buffer (pH 3); recorded after 3 months of storage. C: unfermented apple juice. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

for dextran biosynthesis by *Lb. hordei* TMW 1.1907, as titration of the fruit juices to pH 7 resulted in significant higher dextran yields even upon growth at 20 °C. Dextransucrases from other *Lactobacillus* species typically possess their pH optimum in a moderate acidic environment ranging from pH ~4.0–6.0 (van Hijum et al., 2006; Waldherr et al., 2010). In addition, dextransucrases are released more efficiently at higher pH by other LAB (Otts and Day, 1988). The higher dextran yields obtained in pH adjusted media might therefore be explained by the higher pH causing a more efficient dextransucrase secretion as well as a higher dextransucrase activity. However, dextran production by *Lb. hordei* is not only affected by the pH, but also by the fermentation temperature suggesting that both the cell growth and the environmental pH affect dextran biosynthesis in this strain. The fact that no dextran production took place in any medium at 10 °C is most likely due to the fact that no growth was observed at this temperature. On the contrary, other LAB such as *Lb. sakei* produce more dextran at 10 °C than at 30 °C *via* constitutively expressed, cell-wall anchored dextransucrases. This again implies diverse adaptations towards dextran production among LAB originating from diverse food sources (Prechtl et al., 2018).

Upon growth of *Lb. hordei* TMW 1.1907 in fruit juices, sucrose and glucose concentrations decreased while fructose accumulated in the fermentation media. Therefore, *Lb. hordei* TMW 1.1907 preferentially metabolizes glucose upon growth in fruit juices, while the increasing concentrations of fructose can be explained by the fact that fructose is continuously released in course of e. g. the dextransucrase reactions. Fructose levels at the end of fermentation (48 h) corresponded well to the sum of initial fructose and the fructose liberated from sucrose (Table 1), revealing that solely few or no fructose was consumed over the entire fermentation time.

Malate levels were found to decrease upon fermentation, which is congruent with the presence of a malate transporter (CRI84_05185), malate permease (CRI84_09915) and malic enzyme (CRI84_09410) in the genome of *Lb. hordei* TMW 1.1907 (Accession No: PDDD00000000). The first two enzymes translocate malate over the membrane, while malic enzyme catalyzes the formation of pyruvate from malate. The detected low levels of acetate are in accordance with the presence of a complete phosphoketolase pathway in the genome of *Lb. hordei* (Rouse et al., 2008; Xu et al., 2019; Zheng et al., 2015). The amount of lactic acid produced is comparable to that produced in water kefir fermentations (Laureys and De Vuyst, 2017). However, the detected amount of lactate is significantly higher than that produced in the course of fermentation of other lactic beverages (Malbaša et al., 2008).

Dextran yields in neutralized dilute apple juice (A7) medium were highest at those temperatures, which enabled growth. This is interesting to note, since the utilized apple juice contained lower intrinsic levels of sucrose than the grape juice (see Table 1). This in turn means, that the overall sucrose consumption was higher in grape juice based fermentations as opposed to apple juice based fermentations, in which dextran production was highest. The decrease in sucrose concentration without stoichiometric EPS formation could be explained by the presence of a putative invertase (CRI84_06255) in addition to the putative dextransucrase (CRI84_07775) in the genome of *Lb. hordei* TMW 1.1907. This putative invertase possesses a KxYKxGKxW signal peptide, suggesting cell export and extracellular localization of the protein (Bensing et al., 2014; Bensing et al., 2007). The presence of an extracellular putative invertase suggests that sucrose utilization by *Lb. hordei* TMW 1.1907 might not exclusively take place *via* dextransucrase activity, at which dextransucrases are also known to catalyze sucrose hydrolysis to glucose and fructose without dextran formation (Leemhuis et al., 2013).

In regard to maximum dextran yields, apple juice seems to be a better fermentation substrate for *Lb. hordei* TMW 1.1907 (Fig. 1). However, depending on the product, an apple flavour might be unwanted. In this case, production in G7 medium might be preferable. The growth of LAB in juices has long been studied especially in the context of wine, but also with the goal of producing novel probiotic drinks (Mousavi et al., 2011; Wibowo et al., 1985). The formation of exopolysaccharides in contaminated grape wines is known, but mostly associated with spoilage due to the thickening properties of some of these EPS (Martínez-Viedma et al., 2008; Werning et al., 2006). On the contrary, no thickening of aqueous solutions has been observed for the dextrans produced *in situ* by *Lb. hordei* in the present study. These

results thus demonstrate the possibility to ferment neutralized dilute apple and grape juice for *in situ* production of dextrans exhibiting novel techno-functional properties.

4.3. Characterization of the macromolecular properties and the stability of in situ produced dextrans

The hydrolysis of dextrans under acidic conditions at high temperatures has long been known (Senti et al., 1955). Therefore, the stability of the produced dextrans towards acidic hydrolysis was evaluated as a high level of hydrolysis would lead to a decrease in turbidity forming functionality. Our stability tests revealed that the light scattering and turbidity forming properties of the *in situ* produced dextrans were not strongly affected by pasteurization, which is typically applied in beverage filling plants and further implies the suitability of these molecules regarding their use in beverage related applications. The observed initial drop in optical density (Fig. 4) could be due to a time-dependent dissolving of aggregates present in the freshly fermented juice. The level of turbidity in the solution prepared with isolated dextran (Fig. 4B) was stable throughout the storage experiment and corresponded well to the level of turbidity in the raw fermentate. This clearly shows that the formed dextrans are mainly responsible for the turbidity of the fermented, cell free fruit juices.

The AF4 MALLS data further revealed that the type of fruit juice has a lower impact on the molecular size of dextran molecules than the fermentation temperature. Dextrans produced at 20 °C exhibited a higher molecular weight than those produced at 30 °C. This is in accordance with recent findings reporting the formation of dextran molecules exhibiting comparatively smaller sizes at higher temperatures (Prechtl et al., 2018). In further accordance with the findings of Prechtl et al. (2018), a higher molecular weight of dextran produced by *Lb. hordei* coincided with more intense light scattering in the present work.

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Declaration of Competing Interest

None.

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