Lactic acid bacteria fermentation of brewers´spent grain: assessment of the synthesis *in situ* of dextran

Hanna Nihtilä

Master´s thesis

University of Helsinki

March 2019

HELSINGIN YLIOPISTO — HELSINGFORS UNIVERSITET — UNIVERSITY OF HELSINKI

Tiedekunta — Fakultet/ — Faculty	Masters´s Programme
Faculty of Agriculture and Forestry¤ and Faculty of Biological and Environmental Sciences ¤coordination	Microbiology and Microbial Biotechnology

Tekijä — Författare — Author

Hanna Nihtilä

Työn nimi — Arbetets titel — Title

Lactic acid bacteria fermentation of brewers´spent grain: assessment of the synthesis *in situ* of dextran

Työn laji — Arbetets art — Level	Aika — Datum — Month and year	
Master´s Thesis	March 2019	

Tiivistelmä — Referat — Abstract

Brewers' spent grains (BSG) are by-products of the brewing industry. Utilization of BSG in food applications is challenging, due to its poor technological characteristics. Because of their water retaining properties, interactions with matrix components and impact on texture formation, bacterial exopolysaccharides (EPS) represent a promising tool for improvement of BSG properties. Among bacterial exopolysaccharides, dextran produced *in situ* by lactic acid bacteria (LAB) during fermentation has shown major improvements in technological and sensorial features of products prepared from various types of plant materials. The nutritious composition of BSG may support the growth of LAB and enable *in situ* dextran production.

The aim of this study was to establish and examine the synthesis of dextran by LAB in BSG. Sixteen dextran producing LAB strains were screened for viscosity formation in BSG fermentation. The strains showing the highest viscosity formation were further assessed for fermentation performance. The more suitable fermentation temperature was traced by comparing the viscosifying performance of selected starters at 20 and 25 °C. Dextran amount was determined semi-quantitatively from selected fermented samples showing optimal results, and the presence of oligosaccharides was assessed. Sucrose, glucose, maltose and fructose amounts were analyzed to observe the relation between sugar consumption and dextran and oligosaccharides formation.

Weissella confusa strains A16 and 2LABPTO5 and *Leuconostoc pseudomesenteroides* strain DSM20193 appeared the most promising starters for viscosity formation and thus dextran synthesis in this matrix. From the examined fermentation temperatures, strains showed the highest potential for dextran synthesis at 25 °C. The amount of synthesized dextran ranged from 1.1 to 1.7 % w/w (of the wet weight of the whole sample matrix). The rheological properties of BSG were modified via LAB fermentation and dextran synthesis, resulting in more viscous texture, and its applicability in food systems was thus potentially enhanced.

Avainsanat — Nyckelord — Keywords

Lactic acid bacteria, exopolysaccharides, dextran, brewers' spent grain, fermentation

Säilytyspaikka — Förvaringsställe — Where deposited

https://ethesis.helsinki.fi/en

Muita tietoja — Övriga uppgifter — Further information

Supervision: Rossana Coda

Funded by: Funbrew - Biotransformation of brewers' spent grains: increased functionality for novel food applications ERA-NET Susfood

HELSINGIN YLIOPISTO — HELSINGFORS UNIVERSITET — UNIVERSITY OF HELSINKI

Tiedekunta — Fakultet/ — Faculty	Masters´s Programme
Maatalous-metsätieteellinen tiedekunta[¤] ja bio- ja ympäristötieteellinen tiedekunta [¤]koordinoiva tiedekunta	Mikrobiologian ja mikrobibiotekniikan maisteriohjelma

Tekijä — Författare — Author

Hanna Nihtilä

Työn nimi — Arbetets titel — Title

Olutmäskin maitohappobakteerikäyminen: *in situ* dekstraanisynteesin arviointi

Työn laji — Arbetets art — Level	Aika — Datum — Month and year	
Maisterintutkielma	Maaliskuu 2019	

Tiivistelmä — Referat — Abstract

Ravintorikas mäski on oluentuotannon suurin sivutuote. Olutmäskin hyödyntäminen elintarviketuotannossa on haastavaa sen kehnojen teknologisten ominaisuuksien vuoksi. Bakteerien tuottamat eksopolysakkaridit voisivat mahdollisesti parantaa mäskin ominaisuuksia, sillä ne pystyvät pidättämään kosteutta ja toimimaan vuorovaikutuksessa materiaalin komponenttien kanssa, vaikuttaen siten rakenteen muodostumiseen. Erilaisista viljaraaka-aineista valmistettujen tuotteiden teknologisia ja aistittavia ominaisuuksia onkin pystytty parantamaan esimerkiksi maitohappobakteerien *in situ* -tuottamalla dekstraanilla.

Tämän tutkimuksen tavoitteena oli tarkastella maitohappobakteerien dekstraanisynteesiä mäskissä. 16 dekstraania tuottavaa maitohappobakteerikantaa seulottiin viskositeetin muodostamisen perusteella, ja lupaavimpien kantojen käymisprosessia tarkasteltiin lähemmin kahdessa eri lämpötilassa. Maitohappobakteerien lisääntymistä, materiaalin happamoitumista ja viskositeetin muodostumista verrattiin 20 °C:n ja 25 °C:n lämpötiloissa, joista suotuisampi valittiin korkeamman viskositeetin perusteella. Dekstraani ja oligosakkaridit määritettiin näistä näytteistä. Sakkaroosin, glukoosin, maltoosin sekä fruktoosin määrä analysoitiin sokerinkulutuksen sekä dekstraanin ja oligosakkaridien muodostumisen tutkimiseksi.

Suurimmat viskositeetit saavutettiin *Weissella confusa* -kannoilla A16 ja 2LABPTO5, sekä *Leuconostoc pseudomesenteroides* -kannalla DSM20193, ja ne vaikuttivat siten lupaavilta dekstraanintuottajilta tässä materiaalissa. 25 °C:n käymislämpötilassa viskositeettia muodostui enemmän kuin 20 °C:ssa, joten se arvioitiin suotuisammaksi käymislämpötilaksi halutunlaisen dekstraanin muodostamiselle. Muodostuneen dekstraanin määrä vaihteli 1.1 ja 1.7 % w/w (koko näytteen märkäpainosta) välillä. Olutmäskin muodostui dekstraania, ja sen reologiset ominaisuudet muuttuivat, ja siten sen soveltuvuus elintarvikekäyttöön mahdollisesti parani.

Avainsanat — Nyckelord — Keywords

Maitohappobakteerit, eksopolysakkaridit, dekstraani, olutmäski, mäski, käyminen

Säilytyspaikka — Förvaringsställe — Where deposited

http://www.helsinki.fi/kirjasto/fi/avuksi/yliopiston-julkaisut/e-thesis/

Muita tietoja — Övriga uppgifter — Further information

Ohjaaja: Rossana Coda

Rahoitus: Funbrew - Biotransformation of brewers' spent grains: increased functionality for novel food applications ERA-NET Susfood

Introduction

Brewers' spent grains (BSG) represent the most substantial by-product generated in the brewing industry, with an estimated annual formation reaching ~39 million tons globally [1-3]. Briefly, BSG is the remaining solid fraction formed after the mashing phase in beer production, when the sugar rich wort, further fermented to beer, is separated from the mash [1-3]. This mostly lignocellulosic residue is mainly comprised of barley husks, seed coat and pericarp, along with remnants of the endosperm and fermentable sugars [1-3]. Some variation occurs in the chemical composition of BSG [1, 3, 4]. Generally, it contains abundant amount of fibers, such as lignin, arabinoxylans and β-glucan, and protein, and may carry also other bioactive components with potential benefits, like phenolic compounds with possible antioxidant properties [1, 3, 5-9].

The valuable nutritional profile and generous availability of BSG have been recognized in many publications [1-3]. The current use is mainly focused on animal feed [1-3]. Recently, the interest has raised towards the more efficient exploitation of BSG in food, and it has been suggested that a promising use of BSG could be in baked goods and cereal based products, since they commonly contain whole grains [1, 3, 10]. However, BSG generally has poor technological characteristics, and it is difficult to apply in foods [1-3, 8, 11, 12]. While cereal foods with low supplementations of fiber-rich BSG have occasionally received acceptance, substantial incorporation has often impaired the technological or sensorial quality [1, 8, 12-16]. Suitable technological aids may enable to increase the level of BSG in foods without impairing their desirability. For instance, fermentation technology may allow the utilization of BSG in greater amounts [1, 8, 14, 17]. Among the possibilities brought by this technology, the synthesis of exopolysaccharides (EPS) occurring during fermentation with lactic acid bacteria (LAB) can impart several positive effects to a specific food matrix [18]. Impacts of EPS possibly originate from their water retaining and hydrocolloid properties and possible capacity to form networks and interact with matrix components [18-20].

Dextran is among the most potential bacterial EPS for food use, applied for instance as bodying and thickening agent and as improver of viscosity and moisture retention, as listed by Park and Khan [21]. It is a homoexopolysaccharide, further classified as α-glucan, consisting of D-glucopyranosyl units principally linked via α-1,6-glycosidic bonds [22, 23].

Varying number of different length branches are connected to the backbone via α-1,2, α-1,3 and α-1,4 linkages [22-24]. Dextrans are synthesized extracellularly from sucrose, catalyzed by dextransucrase enzyme [19, 22, 25, 26]. Briefly, during dextran synthesis, the glycosidic bond of sucrose is cleaved, yielding a covalent glucosyl-enzyme intermediate and fructose [22, 23, 25-27]. Glucosyl units are then transferred to the growing dextran molecule, whereas fructose is liberated [22, 23, 25-27]. Dextransucrases are produced by numerous LAB species belonging to the genera *Lactobacillus, Leuconostoc, Pediococcus, Streptococcus,* and *Weissella* [23]. Currently, *Leuconostoc mesenteroides* subsp. *mesenteroides* NRRL B-512F is exploited for commercial dextran production [23, 28, 29].

The properties of dextran molecule, such as molecular weight, frequency and type of the branches and conformation vary, and influence on its impact on the matrix [19-21, 23, 30, 31]. Moreover, dextransucrases differ among bacterial strains, and so does the type of dextran synthesized, thus different interactions with the matrix can occur [20-24, 32]. Dextran of high molecular weight and low level of branches has previously induced positive impacts in cereal applications, for instance in bread baking [20, 33].

Since dextrans perform well in cereal-food applications, they may represent a potential approach for the improvement of the poor structure-forming capacity of BSG, and thus its applicability in foods [20, 30, 31, 33-38]. Additionally, when dextran is produced naturally *in situ* via fermentation by LAB, there is no need to indicate this on the food label [19, 39-41]. This is a positive aspect of this technology, since due to increasing consumer demands for less additives, avoidance of labelling is a desirable approach [19, 40, 41]. Besides EPS synthesis, LAB fermentation may also result in other nutritionally and technologically beneficial modifications of the cereal matrix, such as formation of bioactive compounds, enhancement of antioxidant properties and reduction of anti-nutritional factors [42-46].

LAB generally have demanding nutritional requirements, which may differ between species and even strains [47, 48]. Thus substrate properties largely effect on their performance in the fermentation of certain matrix and, may impact on the dextran synthesis as well [47-50]. The ability of cereal matrices to support the LAB fermentation vary [51]. As listed previously [51-53], the intrinsic factors of cereal substrate, such as the availability and quality of fermentable substrates, vitamins, minerals, nitrogen sources, water-solids ratio and the buffering capacity, influence on the outcome [51-53]. In general, due to high protein, polysaccharide, and mineral content, BSG may appear a potential substrate for LAB fermentation [3, 54, 55]. Additionally, other features of the system affecting dextransucrase

activity, dextran yields and the fermentation process and outcome include: pH, fermentation time and temperature, and sucrose concentration [40, 56-60]. Worth noting, if substrate contains suitable strong acceptors, such as maltose, dextransucrases also mediate oligosaccharide synthesis by transferring the released glucosyl units to this acceptor, rather than to growing dextran molecule, resulting in lower dextran yield [57, 61]. The multifactorial nature of cereal fermentation by LAB highlights the necessity of selecting the suitable starter strains for certain matrix and optimization of culturing conditions, aiming for high yield of desired type of dextran, while minimizing the undesirable effects, such as too intensive acidification with possible negative impacts in some food applications [62, 63].

To the best of my current knowledge (March 2019), *in situ* dextran synthesis by LAB fermentation has never been studied in BSG. *In situ* synthesis of dextran by LAB fermentation represents a potential approach for the improvement of the technological properties of BSG for future food applications. The aim of this study was to establish and examine the synthesis of dextran by LAB in BSG. Viscosity formation during fermentation indicates the presence of large molecules with water binding properties and has been demonstrated to correlate with the *in situ* dextran synthesis [19, 33, 37, 38]. Dextran producing LAB strains previously harnessed for dextran synthesis in cereal matrices, and belonging to genera *Lactobacillus, Leuconostoc* and *Weissella* were screened for *in situ* dextran synthesis in BSG, based on viscosity formation. The most promising strains i.e. showing the highest viscosity, were selected and further screened for their fermentation outcome (growth, acidification, viscosity formation) during experiments at 20 and 25 °C. The more suitable fermentation temperature was traced by comparing the viscosifying performance of the selected starters. Dextran amount was determined semi-quantitatively from selected fermented samples showing optimal results, and the presence of oligosaccharides was assessed. Sucrose, glucose, maltose and fructose amounts were analyzed to observe the relation between sugar consumption, dextran and oligosaccharide formation.

Methods

Raw materials

Brewer's spent grain (BSG) in the present study originated from barley malt extract production and was provided by Viking Malt (Senson, Lahti, Finland). Briefly, at the provider´s site, BSG was separated from the mash, prepared from wet milled malt, mixed with water and mashed with a typical industrial mashing program. The composition of dried BSG as reported by the manufacturer is presented in Table 1. BSG was received frozen and stored at -20 °C. Notably, BSG in this study was in the wet form, and the dry matter content of thawed BSG was 25.2 % w/w, as determined with AACC method 44-15.02. The sucrose used to induce dextran formation in fermentation experiments was common granulated sugar (Rainbow kidesokeri, Suomen Sokeri Oy, Finland). Dry matter contents of sample matrices without sucrose and with sucrose, calculated based on BSG dry matter content, were 10.1 and 13.1 % w/w, respectively.

Table 1. Composition of dried BSG obtained from Viking Malt.

[a] nd refers to not detected

Dry matter %	96.7
Energy /100 g	841 kJ/ 201 kcal
Protein %	19.8 %
Ash g/100 g	2.9
Fat g/100 g	9.3
Dietary fiber g/100 g	55.3
Carbohydrates g/100 g	9.4
Sugars g/100 g	nd [a]
Maltose g/100 g	nd [a]
Glucose g/100 g	nd [a]
Fructose g/100 g	nd [a]
Maltotriose g/100 g	nd [a]
Sodium mg/100 g	8.1
Potassium mg/100 g	110
Calcium mg/100 g	140
Zink mg/100 g	9.7
Magnesium mg/100 g	210

Lactic acid bacteria strains and culturing

The list of screened lactic acid bacteria (LAB) strains is presented in Table 2. Strains were stored in cryo-tubes with glycerol as cryo-protectant (200 µl of 99.5 % glycerol (AnalaR NORMAPUR® ACS, VWR BDH Chemicals, UK) and 800 µl of 24 h culture) at -80 °C and routinely cultured in MRS broth (de Man, Rogosa & Sharpe Broth, LABM, UK) at 30 °C.

Table 2. List and characteristics of the LAB strains used in this study.

Specimen	Strain Code	Source	Type strain (Yes/No)	Culture collection
Lactobacillus fermentum	4Rwb	African grains	No	University of Helsinki, Department of Food and Nutrition, own collection
L. mesenteroides subsp. Mesenteroides	LMG 7939	Slime on root beer	No	BCCM/LMG, Ghent University, Belgium
L. mesenteroides	I21	Faba bean	No	University of Helsinki, Department of Food and Nutrition, own collection
L. mesenteroides	DSM20343	Fermented olives	Yes	Leibniz Institute - German Collection of Microorganisms and Cell Cultures, Germany
Leuconostoc pseudomesenteroides	DSM20193	Cane juice	Yes	Leibniz Institute - German Collection of Microorganisms and Cell Cultures, Germany
Leuconostoc citreum	DSM5577	Honeydew or rye ear	Yes	Leibniz Institute - German Collection of Microorganisms and Cell Cultures, Germany
Weissella cibaria	Sj1b	Onion powder	No	Division of Food Hygiene and Environmental Health, University of Helsinki
W. cibaria	LMG 17699	Chili bo (Malaysian food)	Yes	BCCM/LMG, Ghent University, Belgium
Weissella confusa	Sj5-4	Onion powder	No	Division of Food Hygiene and Environmental Health, University of Helsinki
W. confusa	Sj28-1	Onion powder	No	Division of Food Hygiene and Environmental Health, University of Helsinki
W. confusa	C2	African grains	No	University of Helsinki, Department of Food and Nutrition, own collection
W. confusa	5E7	Wheat sourdough	No	University of Helsinki, Department of Food and Nutrition, own collection
W. confusa	A16	African grains	No	University of Helsinki, Department of Food and Nutrition, own collection
W. confusa	2LABPTO5	African grains	No	University of Helsinki, Department of Food and Nutrition, own collection
W. confusa	DSM20194	Soured carrot mash	No	Leibniz Institute - German Collection of Microorganisms and Cell Cultures, Germany
W. confusa	NEY6	Sourdough	No	Department of Soil, Plant and Food Science, University of Bari, Italy

Sample preparation and fermentation

BSG was thawed overnight at 4 °C and warmed up at room temperature for 45-60 min during sample preparation. The BSG-water ratio of 1:1.5 was selected based on pre-trials (data not shown), to achieve measurable consistency for viscosity assessment (Table 3). To enable dextran synthesis, 10 % w/w of BSG was substituted with sucrose, resulting in initial sucrose content of 4 % w/w of total wet weight of sample. Sucrose content was selected based on previous studies, in which sucrose supplementation has generally been 10 % of flour weight, corresponding to 2-4 % w/w of wet weight [33, 37, 38, 49]. Fermented samples with sucrose supplementation are henceforth referred as EPS+, and counterparts without sucrose supplementation, as EPS-.

Table 3. Recipes for sample matrices presented as percentages of wet weight.

Ingredient	Without sucrose (% w/w)	With sucrose (% w/w)
BSG	40	36
Distilled water	60	60
Sucrose	-	4
Total sample matrix	**100**	**100**

Cell density of 24-h broth culture was estimated based on optical density (OD_{600}), measured with Novaspec II visible spectrophotometer (Amersham Pharmacia Biotech, UK). Targeted initial cell density of the starter was ca. 6 $\log_{10}$ cfu g^{-1} which was achieved with 0.3 V/w % inoculum of 24 h culture, containing ca. 9 $\log_{10}$ cfu g^{-1}. Cells were harvested with 10 min centrifugation at 9600 g and supernatant was discarded. Cell sediment was suspended in an aliquot of the water needed for the experiment and inoculated in the matrix.

Samples were fermented for 24 h at 25 or 20 °C. In detail, in the initial screening phase to determine whether viscosity formation was induced, samples were fermented at 25 °C. In the second phase with the selected starters, with the aim of assessing the effect of temperature on fermentation outcome and dextran formation, fermentation was performed at both 20 °C and 25 °C. Additionally, BSG samples, prepared as previously described without LAB inoculum, were incubated in the above conditions and used as controls. Two biological replicates were prepared for the initial screening trials, and two or three for the characterization trials. Samples were collected before and after fermentation and analyzed directly (pH,

viscosity, microbial enumeration), stored at -20 °C (total titratable acidity) or freeze dried (dextran, oligosaccharides, sugars) for further analyses.

Acidification properties

Acidification was assessed by pH and total titratable acidity (TTA) measurements before and after fermentation, following the methods commonly exploited in cereal fermentation trials [33, 38, 49, 64]. Knick (Germany) Portamess 752 Calimatic pH meter and BlueLine 11 pH electrode by SI analytics (Germany) were used for pH measurement. TTA was measured by titrating 10 g of melted sample with 0.1 M NaOH until pH 8.5 was reached, as described previously by Katina et al. [64]. Before TTA measurement, samples were homogenized by grinding with mortar and pestle for 3 minutes, followed by blending with 95 ml of Milli-Q water and 5 ml of acetone for 1.5 minutes. TTA values were reported as ml of 0.1 M NaOH consumed. Measurements were carried out in duplicate.

Viscosity measurements

The viscosity of the samples was evaluated both visually and instrumentally. Viscosity was assessed visually on a scale 0-5, in which 0 was considered to correspond to equal or lower viscosity as before fermentation, whereas 5 corresponded to the most viscous fermented sample. Measurement was performed with rotational rheometer (Rheolab QC, Anton Paar, Germany), using C-CC27 measuring cup, and ST 22.02-4V probe, at 22 °C, following a protocol adapted from a previous study by Xu et al. [65]. Viscosity was analyzed with shear rates increasing from 2 s^{-1} to 100 s^{-1}, followed by decreasing back to 2 s^{-1}. Viscosities (Pa·s) at shear rate 100 s^{-1} were compared. Measurements were performed in duplicate.

Microbial enumeration

Microbiological enumeration was carried out by classical plate counting techniques. The number of total aerobic mesophilic microbes, presumptive LAB, *Bacillus cereus*, *Enterobacteriaceae*, as well as yeasts and molds, was analyzed before and after fermentation. Serial dilutions in physiological salt solution (0.9 % w/V NaCl) were performed, followed by culturing on suitable medium and colony counting. Total mesophilic microbes were counted on PCA, presumptive LAB on MRS agar and *Enterobacteriaceae* on VRBGA (Table 4). Yeasts and molds were enumerated on YPD [66] and malt extract agar, respectively. YPD and malt extract agar were supplemented with chloramphenicol to suppress bacterial growth. When necessary, Leica DM1000 LED microscope was used for cell morphology examination to confirm the distinguishment of yeast and mold colonies from bacterial colonies on these plates. *B. cereus* were enumerated on PEMBA supplemented as recommended by the manufacturer, by the recognizable blue white halo colonies. All enumerations were performed in duplicate.

Table 4. Culturing media and conditions used as described by the manufacturer for microbiological enumeration of BSG before and after the fermentation process.

Group of interest	Medium	Manufacturer	Plating method	Incubation temperature (°C)	Incubation time (h)
Lactic acid bacteria	de Man, Rogosa and Sharpe agar (MRS agar)	LABM, UK	Pouring	30	48
Total mesophilic microbes	Plate Count agar (PCA)	LABM	Pouring	30	72
B. cereus	*Bacillus cereus* medium (PEMBA)	LABM	Spreading	30	24
	+ Polymyxin B	LABM			
	+ Egg yolk emulsion	LABM			
Enterobacteriaceae	Violet Red Bile Glucose Agar (VRBGA)	LABM	Pouring	37	24
Yeasts	Yeast extract - peptone -dextrose agar (YPD) [66]		Spreading	25	72
	Ingredients: yeast extract, bactopeptone, dextrose, agar	LABM			
	+ Chloramphenicol 0.01 %	Oxoid, UK			
Molds	Malt extract agar + Chloramphenicol 0.01 %	LABM Oxoid	Spreading	25	120

Dextran, oligosaccharides and sugar analysis

Dextran, oligosaccharides, sucrose, maltose, glucose and fructose amounts were determined from freeze dried samples of BSG fermented at 25 °C with selected starters and from unfermented sample (control) without sucrose supplementation.

Dextran was extracted using an enzyme assisted method described by Katina et al. [33], in which dextran is hydrolyzed to glucose which is then quantified. Used dextranase was from *Chaetomium erraticum* (D0443-50ML, Sigma-Aldrich, Germany), transglucosidase

from *Aspergillus niger* (E-TRNGL Megazyme, Ireland), and pure dextran used for determining the recovery rate from *Leuconostoc* spp. (95771-10G, Sigma-Aldrich). Based on pre-trial (data not shown), dextran recovery from BSG matrix was ca. 67 %, thus the correction factor 1.5 was used to approximate the amount of formed dextran.

For sugar and oligosaccharides analyses, 200 mg of sample was mixed with 5 ml MilliQ-water to dissolve the sugars, followed by 5 min boiling in order to inactivate the endogenous enzymes. Supernatant was recovered by 15000 g 10 min centrifugation, filtered through Amicon Ultra-0.5 centrifuge filters (Millipore, Billerica, MA), and used for determination.

Determinations of glucose released from dextran hydrolysis, and other sugars were carried out with high performance anion exchange chromatography with pulse amperometric detection (HPAEC-PAD) system as described previously by Wang et al. [37]. Sucrose, glucose, maltose and fructose (Merck, Germany) were used as standards, and 2-deoxy-galactose as an internal standard. Dionex CarboPac PA1 Analytical (4 × 250 mm, Thermo Scientific) was used as a column, Waters 2465 as a pulse amperometric detector, Waters 2707 as an autosampler, and three Waters 515 as HPLC pumps (Waters, USA). Milli-Q water and 0.2 M NaOH were used as eluents, and the flow rate was 1.0 ml min^{-1}. Measurements were performed in duplicate. Results were reported as % w/w of the wet weight of the whole sample matrix.

Oligosaccharides were determined with HPAEC-PAD as described by Katina et al. [33]. Glucose, sucrose, fructose, maltose (Merck) were used as standards. Dionex CarboPac PA100 Analytical (4 × 250 mm, Thermo Scientific) was used as a column, two Waters 515 as HPLC pumps (Waters), Waters 717 as autosampler, and Decade detector with a gold electrode as a detector (Antec Leyden, The Netherlands). In the eluation, 100 mM NaOH-1 M NaOAc gradient was used at a flow rate of 1 ml min^{-1}. Determinations were performed in duplicate.

Statistical analysis

Statistical significance of differences between the obtained results was examined with IBM SPSS Statistics 25, using One-way ANOVA and Tukey's test ($p < 0.05$). Two biological replicates were prepared for the screening trials, and two or three for the optimization trials. The results are presented as mean values of biological replicates.

Results

Fermentation experiments: Screening and selection of LAB strains

Acidification

After 24 h of fermentation at 25 °C, initiated by the LAB starters reported in Table 2 singly inoculated in BSG, acidification occurred in all the BSG samples, and pH decreased from 6.3 to 3.9-5.7 (Table 5). In most of the cases, no significant differences in pH values between EPS+ and their EPS- counterparts were observed, with a few exceptions. *W. cibaria* Sj28-1 and *L. mesenteroides* DSM20343 EPS+ samples showed significantly lower pH compared to their EPS- analogues.

Table 5. pH values of fermented BSG samples (fermented at 25 °C). Initial row refers to the value before fermentation. EPS- and EPS+ refer to BSG samples fermented without and with sucrose supplementation, respectively.

[a-j] Values with different superscript letters are significantly different ($p < 0.05$), n = 2-3.

Sample	**pH**	
	EPS-	EPS+
Initial		
Average	6.3 ± 0.1 [a]	6.3 ± 0.1 [a]
Fermented at 25 °C		
LMG7939	5.0 ± 0.0 [f, g, h, i]	5.4 ± 0.0 [i, j]
DSM20193	3.9 ± 0.1 [b]	4.2 ± 0.2 [b, c, d]
Sj1b	4.8 ± 0.1 [d, e, f, g, h]	5.2 ± 0.1 [g, h, i, j]
LMG17699	4.7 ± 0.1 [c, d, e, f, g, h]	5.0 ± 0.1 [f, g, h, i]
Sj5-4	5.0 ± 0.0 [f, g, h, i]	4.6 ± 0.3 [c, d, e, f, g]
Sj28-1	5.7 ± 0.0 [j]	4.7 ± 0.0 [c, d, e, f, g, h]
DSM5577	5.2 ± 0.1 [g, h, i, j]	4.7 ± 0.0 [c, d, e, f, g, h]
C2	4.9 ± 0.0 [f, g, h, i]	4.7 ± 0.1 [c, d, e, f, g, h]
5E7	4.2 ± 0.4 [b, c, d, e]	4.0 ± 0.2 [b]
A16	4.6 ± 0.2 [c, d, e, f, g]	4.8 ± 0.1 [e, f, g, h, i]
2LABPTO5	4.4 ± 0.2 [b, c, d, e, f]	4.8 ± 0.2 [d, e, f, g, h]
DSM20194	5.0 ± 0.0 [g, h, i]	4.9 ± 0.0 [f, g, h, i]
I21	5.3 ± 0.2 [h, i, j]	4.8 ± 0.1 [d, e, f, g, h]
DSM20343	5.4 ± 0.0 [i, j]	4.7 ± 0.0 [c, d, e, f, g, h]
4Rwb	4.2 ± 0.1 [b, c]	4.0 ± 0.1 [b]
NEY6	4.2 ± 0.1 [b, c, d]	4.2 ± 0.1 [b, c, d, e]

Viscosity formation

Fresh BSG consisted of small grain particles and showed incoherent texture, with phase separation occurring in samples rapidly after mixing. Before fermentation, viscosity was not remarkably different between samples with and without sucrose supplementation.

After fermentation at 25 °C, the instrumental viscosity values ranged from 0.1 to 3.1 Pa·s (Figure 1, Table S1, supplementary material). From examined samples, EPS+ fermented by DSM20193, 5E7, A16, 2LABPTO5 and NEY6 exhibited the largest differences from unfermented sample in the instrumental measurement. Minor viscosity formation was observed in EPS+ samples fermented by LMG17699, Sj-28-1, DSM20194 and DSM20343 as

well, scoring 0.5-1 in visual assessment, but remaining just barely detectable in the instrumental measurement. The highest viscosities in the instrumental measurement, with statistical significance, were obtained from EPS+ samples fermented by *W. confusa* strains 2LABPTO5 and A16, and *L. pseudomesenteroides* strain DSM20193, estimated also visually with a score of 5. None of the EPS- samples showed significant increase in viscosity during fermentation. Furthermore, phase separation after mixing started to occur rapidly after fermentation in EPS- samples, while it was absent during the visual observation in viscous fermented EPS+ samples (Figure 2).

Based on the results of this screening, *W. confusa* strains A16 and 2LABPTO5 as well as *L. pseudomesenteroides* DSM20193, showing the highest viscosity increase due to sucrose supplementation, were selected for further trials.

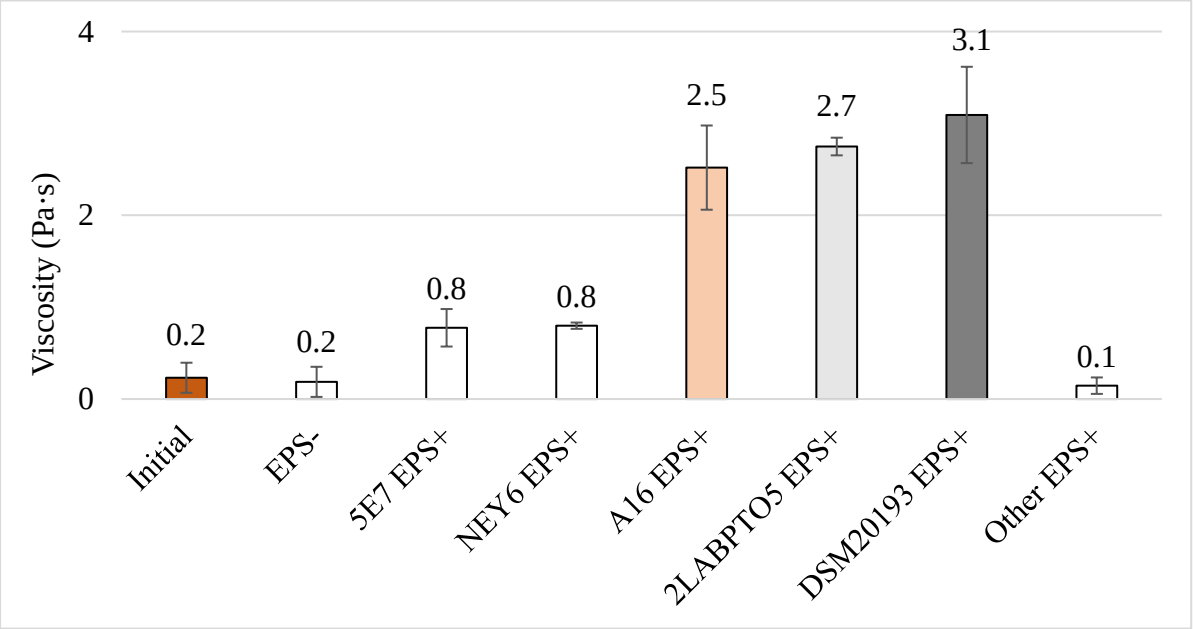

Figure 1. Viscosity values as determined by Rheolab QC (Pa·s at shear rate 100 s^{-1}), from BSG samples before fermentation (Initial), from EPS- samples, from representative samples showing the highest change in the viscosity after fermentation and from other EPS+ samples.

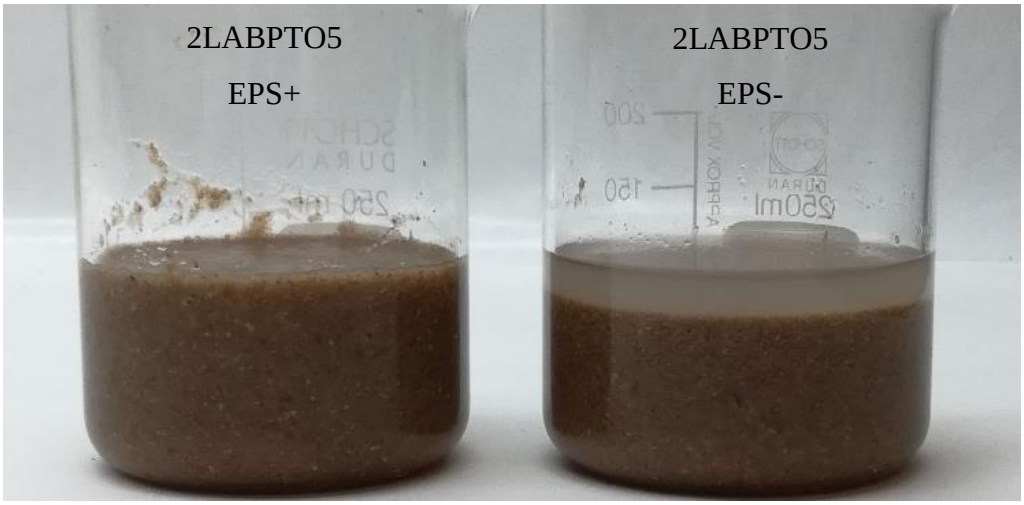

Figure 2. Representative image of dextran synthesis during LAB fermentation. Samples fermented by *W. confusa* 2LABPTO5 5 minutes after mixing. EPS+ refers to sample fermented with sucrose supplementation, EPS- to sample without sucrose supplementation.

Fermentation experiments: Assessment of selected starters performance at 20 and 25 °C

Acidification properties

Before fermentation, pH of BSG sample matrix was 6.3 and TTA 1.0 ml (Figures 3 and 4). In all trials with selected starter strains, BSG was acidified during fermentation. In general, *W. confusa* A16 and 2LABPTO5 showed similar results in both temperatures, with slightly, but not significantly, higher TTA (3.2-3.9 ml) and lower pH (4.4-4.6) in EPS- than EPS+ (2.5-3.1 ml, 4.6-4.8) samples. The lowest pH values (3.9-4.2) and the highest TTA values (5.4-6.6 ml) were achieved with *L. pseudomesenteroides* DSM20193, with an exception of EPS- sample fermented at 20 ℃, which showed pH 4.5 and TTA 3.7 ml. Spontaneous fermentation resulted in pH values varying from 5.0 to 6.3, without notable differences between the two fermentation temperatures.

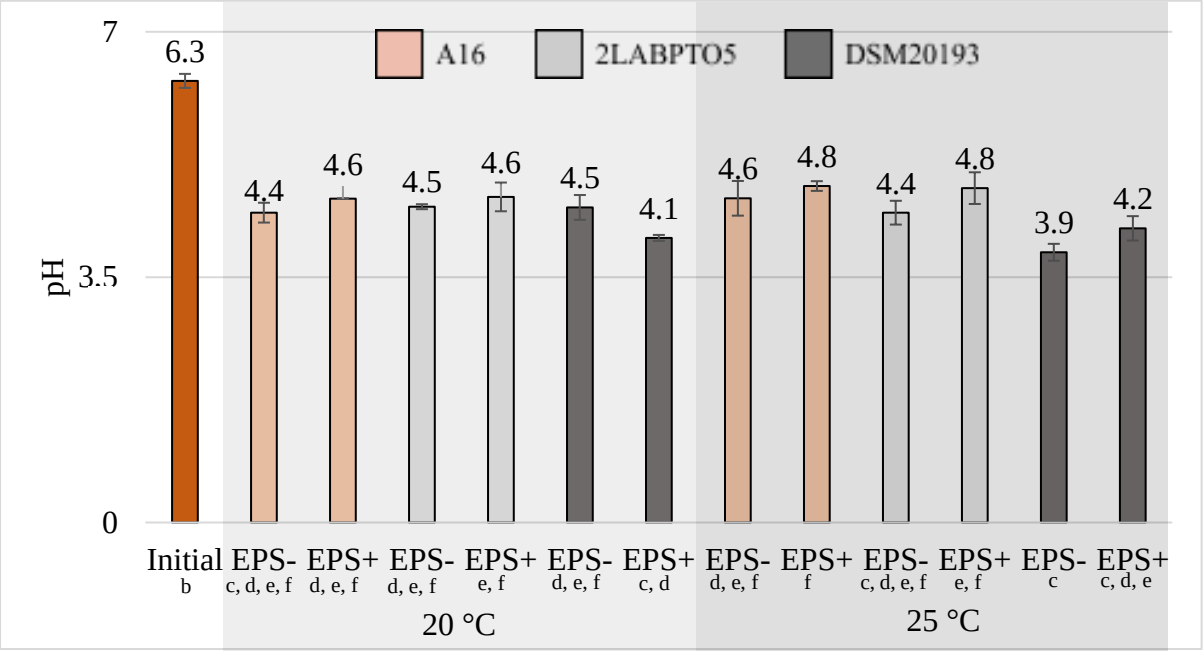

Figure 3. pH values before (Initial) and after 24 hours of fermentation. EPS- and EPS+ refer to fermented samples prepared without and with sucrose supplementation, respectively.

[b-f] Values with different superscript letters are significantly different ($p < 0,05$), n = 2-3.

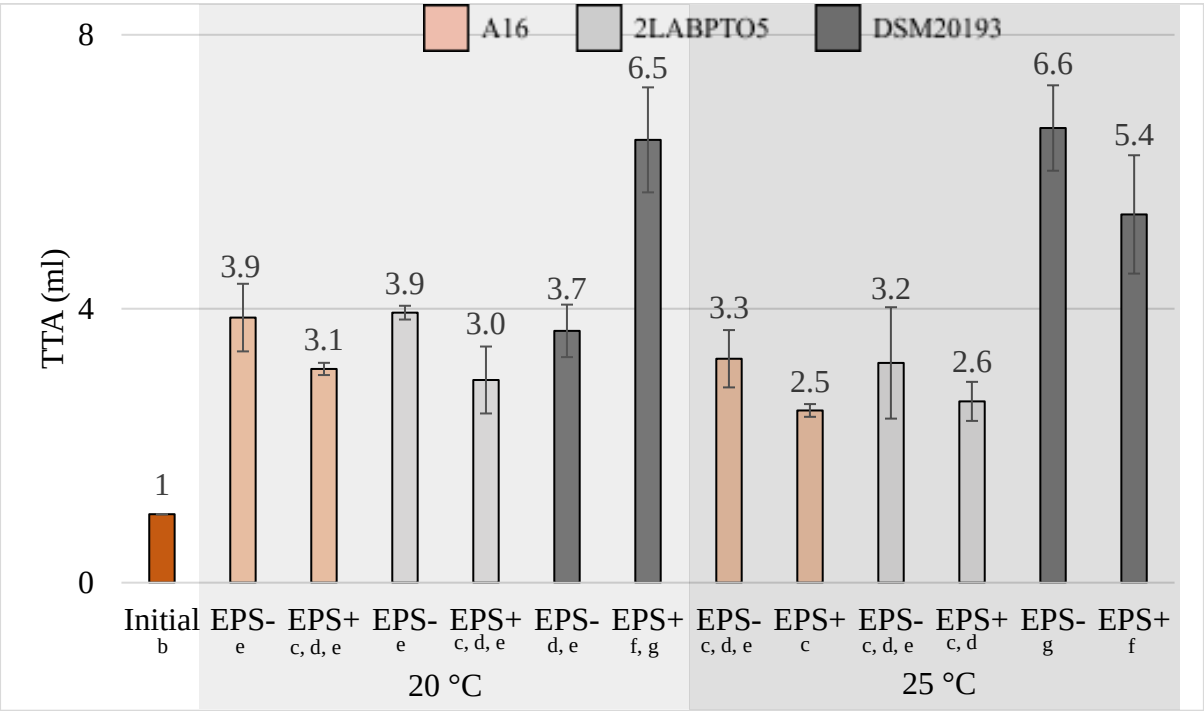

Figure 4. TTA values before (Initial) and after 24 hours of fermentation. EPS- and EPS+ refer to fermented samples prepared without and with sucrose supplementation, respectively.

[b-g] Values with different superscript letters are significantly different ($p < 0.05$), n = 2-3.

Viscosity formation

As expected based on the preliminary screening trials, viscosity of any of the EPS- samples did not differ significantly from initial situation, independently of the temperature tested. In these cases, the instrumental viscosity value settled at 0.1-0.3 Pa·s at shear rate 100 s^{-1}. EPS+ DSM20193 and 2LABPTO5 showed higher viscosity at 25 °C in instrumental and visual assessment, whereas A16 appeared more viscous as well, but difference in the viscosity values did not reach statistical significance (Figure 5). Viscosity increase was substantial in EPS+ samples also when fermentation was performed at 20 °C and scored 4 in visual assessment. Moreover, according to visual observation, 2LABPTO5 and A16 EPS+ sample mass appeared more elastic and slightly less thick than corresponding DSM20193 after fermentation at both conditions. Since the highest viscosity values were observed after fermentation at 25 ℃, these samples were selected for further dextran and sugar analyses.

No viscosity increases or delay in phase separation occurred in any of the

spontaneously fermented samples with or without sucrose, and instrumentally determined viscosity was 0.2 Pa·s at shear rate 100 s^{-1}.

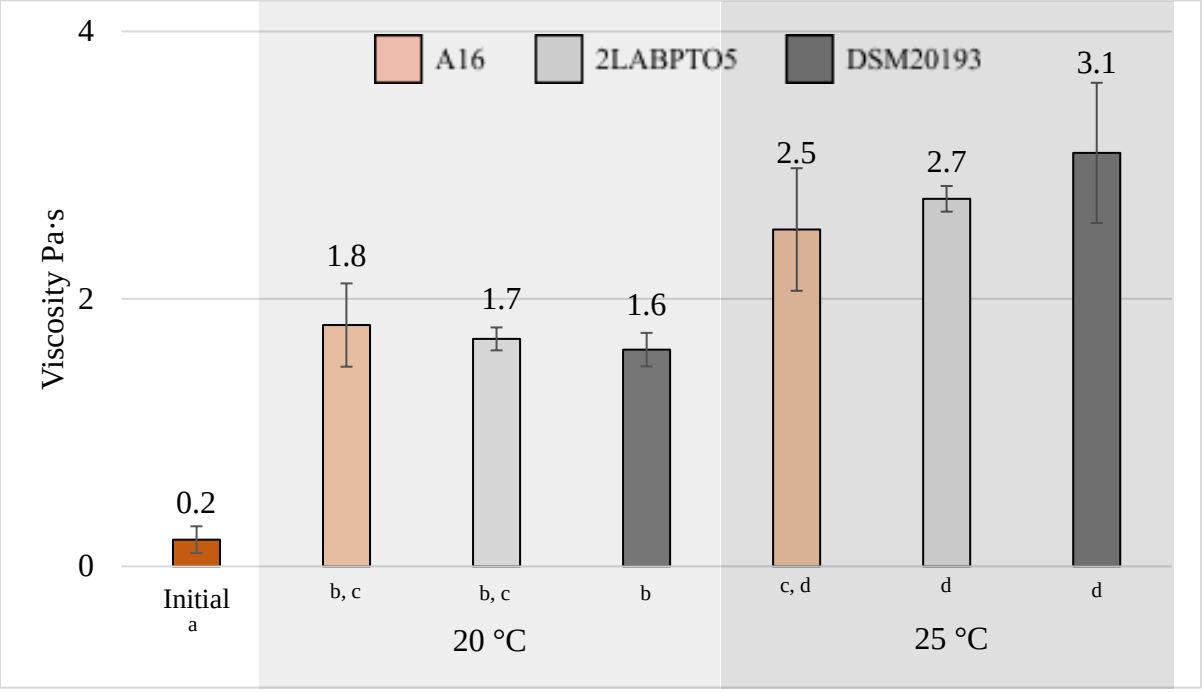

Figure 5. Viscosity values as determined by Rheolab QC (Pa·s at shear rate 100 s^{-1}) of unfermented sample (Initial) and EPS+ (fermented sample with sucrose supplementation) samples fermented at two different temperatures.

[a-d] Values with different superscript letters are significantly different ($p < 0.05$), n = 2-3.

Microbiological enumeration

The number of presumptive LAB in not fermented fresh sample matrix varied between 0 and 2 $\log_{10}$ cfu g^{-1} and the number of total mesophilic microbes between 2 and 3 $\log_{10}$ cfu g^{-1}. Yeasts, molds, *Enterobacteriaceae* or *B. cereus* were never detected from unfermented sample matrices. Sucrose supplementation did not affect the microbial density in the fresh matrix. Increase of presumptive LAB cell density was found in all the fermented samples (Table 6). In the trials carried out with the selected *W. confusa* starter strains, final LAB cell density was generally slightly higher after fermentation at 20 °C than at 25 °C. In the these samples, cell

density was similar among EPS+ and EPS- counterparts, increasing ca. 2.7-2.9 log cycles during fermentation at 20 ℃, and 2.4 at 25 ℃. Contrarily, DSM20193 showed some differences between EPS+ and EPS- samples. At 20 ℃ EPS+ sample showed an increase of 2.6 cycles, and the EPS- one only 2.3. On the contrary, at 25℃, the EPS+ one showed less increase, 2.3 cycles, and the EPS- one more, 2.6. Total mesophilic count followed the same pattern as the presumptive LAB count. *Enterobacteriaceae*, *B. cereus*, yeasts or molds were never found from samples fermented by selected starter strains.

The development of microbial community during spontaneous fermentation of BSG appeared different: the variation in cell density and microbial diversity between samples taken from different parts and batches of BSG was extensive, whereas sucrose supplementation did not result in significant differences. After spontaneous fermentation at 20 or 25 °C, presumptive LAB count reached 4-8 $\log_{10}$ cfu g^{-1} and total mesophilic count 5-8 $\log_{10}$ cfu g^{-1}. *B. cereus* was never found after fermentation at 20 °C, but always after fermentation at 25 °C, at a cell density varying from 1 to 4 $\log_{10}$ cfu g^{-1}. *Enterobacteriaceae* appeared occasionally after fermentation at 25 °C, number of colony forming units varying from 4 to 7 $\log_{10}$ cfu g^{-1}, and never after fermentation at 20 °C.

Noteworthy, despite of chloramphenicol supplementation, a few colonies, confirmed as bacterial by microscopy, appeared on YPD and malt extract agar plates, which were intended for yeast and mold enumeration.

Table 6. Cell density (log_{10} colony forming units g^{-1}) of lactic acid bacteria and total mesophilic microbes before (initial) and after fermentation by selected starters *W. confusa* strains A16 and 2LABPTO5, and *L. pseudomesenteroides* strain DSM20193. EPS- and EPS+ refer to BSG samples fermented without and with sucrose supplementation, respectively.

[a-g] Values in the same group with different superscript letters are significantly different ($p < 0.05$), n = 2-3.

	Lactic acid bacteria (log_{10} cfu g^{-1})		**Total mesophilic microbes (log_{10} cfu g^{-1})**	
	EPS-	EPS+	EPS-	EPS+
Initial				
A16	6.1 ± 0.1 [a]	6.2 ± 0.1 [a]	6.1 ± 0.2 [a, b]	6.1 ± 0.1 [a]
2LABPTO5	6.1 ± 0.1 [a]	6.2 ± 0.1 [a]	6.1 ± 0.0 [a]	6.2 ± 0.1 [a]
DSM20193	6.6 ± 0.1 [b]	6.6 ± 0.0 [b]	6.6 ± 0.1 [b]	6.6 ± 0.2 [b]
Fermented at 20 °C				
A16	9.1 ± 0.2 [e, f, g]	9.0 ± 0.0 [e, f]	8.9 ± 0.3 [e, f]	8.9 ± 0.0 [e, f]
2LABPTO5	9.0 ± 0.0 [e, f, g]	8.8 ± 0.1 [d, e]	9.0 ± 0.0 [e, f]	8.9 ± 0.2 [d, e]
DSM20193	8.9 ± 0.0 [e, f]	9.3 ± 0.1 [g]	8.9 ± 0.0 [e, f]	9.2 ± 0.1 [f]
Fermented at 25 °C				
A16	8.5 ± 0.0 [c]	8.6 ± 0.0 [c, d]	8.4 ± 0.1 [c]	8.5 ± 0.0 [c]
2LABPTO5	8.5 ± 0.1 [c]	8.5 ± 0.1 [c]	8.4 ± 0.1 [c]	8.5 ± 0.1 [c, d]
DSM20193	9.2 ± 0.1 [f, g]	8.9 ± 0.1 [e]	9.3 ± 0.1 [f]	8.9 ± 0.1 [d, e]

Dextran, oligosaccharides, and sugar determination

Before fermentation, uninoculated BSG matrix without sucrose supplementation had 0.1 ± 0.0 % w/w (of the wet weight, henceforth) glucose, 0.3 ± 0.0 % w/w maltose, and no intrinsic fructose, sucrose, dextran or oligosaccharides were detected. As mentioned above, sugar supplementation was 4 % w/w. In EPS+ samples, the supplemented sucrose was completely consumed during fermentation. Intrinsic maltose and glucose were consumed, and dextran, fructose and oligosaccharides were formed in all EPS+ samples (Figure 6), while no fructose, oligosaccharides or dextran were found in EPS- samples.

1.8 ± 0.1, 1.9 ± 0.1, and 1.2 ± 0.2 % w/w of fructose was liberated in EPS+ A16,

2LABPTO5 and DSM20193 samples, respectively. A16 and 2LABPTO5 EPS+ samples showed similar dextran quantity (1.7 ± 0.0 and ± 0.1 % w/w, respectively), which was higher than of DSM20193 EPS+ sample (1.1 ± 0.2 % w/w). Based on the retention time and presence of maltose acceptors in the substrate, formed oligosaccharides were isomaltooligosaccharides. Currently, isomaltooligosaccharides cannot be quantified due to lack of suitable standards and only a qualitative analysis can be provided.

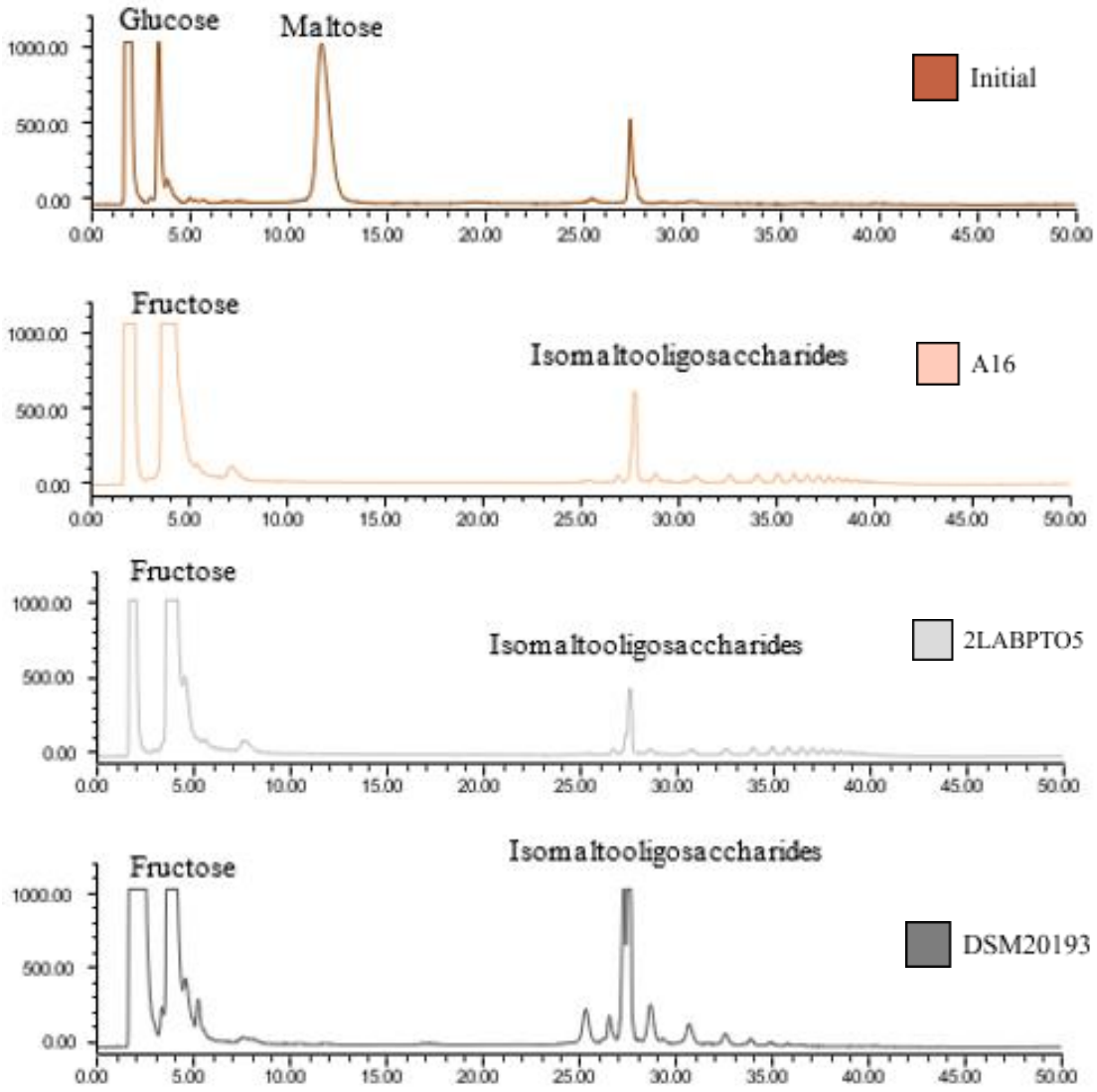

Figure 6. HPAEC-PAD chromatograms of representative EPS+ (fermented sample prepared with sucrose supplementation) samples after fermentation at 25 °C and before fermentation without sucrose supplementation (Initial) from oligosaccharide analysis.

Discussion

Screening and selection of LAB strains

In this study, BSG was utilized as a fermentation matrix for EPS synthesis by LAB. First, different LAB strains were screened for their capacity to produce EPS in BSG, assessed by viscosity formation and acidification in the fermented matrix.

A trend of similar pH of EPS+ and EPS- sample occurred in screening trials, with a few exceptions showing higher acidification in the EPS+ counterpart. This may be due to different preferences of certain strains to ferment the few sugars available (i.e. glucose and maltose) or potentially deriving from hydrolysis of BSG carbohydrates [47, 48, 51]. It can be hypothesized that sucrose supplementation may have facilitated the fermentation of the strains for which the intrinsic fermentable substrates available in BSG were not usable. Indeed, the interactions of cereal matrix and microbial community were described by Gänzle [67] as interdependent, in other words, metabolic products of microbes have an impact on the cereal components and, vice versa. For instance, the acids produced by microorganisms may affect the activity of grain enzymes, which as a consequence create substrates for microbes [67]. Because the BSG in this study was not heat treated or sterilized, endogenous enzymatic activities of the grains, as well as the microbial activity, may have impacted on the fermentation kinetics, for instance by liberating fermentable sugars during the trial [1, 68]. Notably, intrinsic glucose and maltose amounts in BSG observed in this study slightly differed from that provided by the manufacturer, which might due to a different analytical method.

The physiological importance of EPS for bacteria is not entirely known, and various factors appear to influence on the nature and efficiency of dextran synthesis, as described above [19, 47, 48, 51, 56, 57, 61]. In turn, molecular properties largely affect the functionality of EPS [19, 21, 30]. Besides structural diversity of dextran molecules synthesized by different LAB species and strains, also their interaction with the matrix components likely effects on the viscosity [19, 21, 23, 24, 32]. Increased viscosity values generally indicate the presence of high molecular weight compounds with water binding capacity and viscosity increase due to *in situ* dextran synthesis has been well demonstrated in numerous studies [18, 33, 37, 65]. Therefore, assessment of increase in the viscosity can be exploited as an indicative

parameter for high molecular weight dextran synthesis during LAB fermentation although the viscosity values do not directly correspond to the amount of dextran formed. Based on the viscosity increase, the strains, *L. pseudomesenteroides* DSM20193, *W. confusa* A16 and 2LABPTO5, were considered the best performing candidates.

Assessment of selected starters performance at 20 and 25 °C

Weissella spp. and *Leuconostoc* spp. are facultatively anaerobic and heterofermentative bacteria, producing lactic acid, CO_2, ethanol and/or acetate by fermenting glucose via 6-phosphogluconate/phosphoketolase pathway [28]. Optimal growth temperature for *Leuconostoc* spp. is reported to be 20-30 °C, but most species, including *L. pseudomesenteroides*, are also capable of growing at temperatures as high as 37 °C [28]. *Weissella* spp. may grow between 15 and 37 °C, and some even at 42-45 °C, including strains of *W. confusa* [28, 69, 70]. In this study, the temperatures of 20 and 25 °C were selected aiming of allowing the mild acidification of the substrate. In fact, de Vuyst and Degeest [71] stated that suboptimal incubation temperature appears to enhance the EPS synthesis by mesophilic LAB. Moreover, application-wise, the ability to grow in low temperature is considered a preferable property of a starter in cereal applications [72].

In the present study, acidification in *W. confusa* A16 and 2LABPTO5 samples did not differ significantly between the two temperatures examined. On the contrary, *L. pseudomesenteroides* DSM20193 EPS- samples fermented at 20 °C showed considerably lower acidity than the EPS+ one, while sucrose supplementation did not induce the acidification at 25 °C. This may indicate that additional sucrose supported the metabolism of DSM20193 in suboptimal conditions at 20 °C. Greater sucrose utilization for growth and acidification may, in turn, have resulted into lower amount of sucrose available for dextran synthesis, which could partially explain the lesser viscosity formation in this lower temperature, in the case of this strain in particular.

Acidification by LAB in cereal fermentations varies among strains and substrates, and same strains have produced acids differently in diverse matrices [33, 49, 50]. Samples fermented by *W. confusa* strains A16 and 2LABPTO5 had generally lower acidity than corresponding samples fermented by *L. pseudomesenteroides* strain DSM20193, after fermentation at 25 °C and with the exception of EPS- DSM20193, also at 20 °C. The

explanation for more intensive acidification in *L. pseudomesenteroides* DSM20193 samples in comparison to *W. confusa* A16 and 2LABPTO5, is presumably complex and due to different factors. Higher acidification by *Leuconostoc* spp. than *Weissella* spp. in same matrix has been reported also earlier [33, 65]. Notably, *Leuconostoc* spp., like the majority of heterofermentative LAB, may reduce fructose to mannitol, catalyzed by mannitol dehydrogenase, with associated acetate, instead of ethanol, formation [28, 73]. It has been observed previously, that *Weissella* spp. typically do not possess such activity, thus lacking the concomitant acetate formation, and commonly result in low acetate production [33, 36, 49, 74-77]. Fructose liberated from sucrose by dextransucrase could therefore induce the acetate formation by *L. pseudomesenteroides* DSM20193, as also previously observed in faba bean fermentation [65]. Noteworthy, sucrose supplementation did not result in higher TTA in faba bean trials with DSM20193, but still led to slightly higher acetate and lower lactic acid content [65], which indicates that variations in lactic and acetic ratio may not reflect on pH and TTA values.

Nonetheless, TTA values of all the fermented samples in this study can be considered generally quite low, compared to previous observations in BSG or in other cereal substrates [8, 14, 33, 49, 50]. Stronger acidification (pH 3.68 and 4.07, TTA 14.72 and 9.7 ml, respectively) was reached in previous studies in BSG sourdough fermentation, although fermentation conditions and starter cultures were different [8, 14]. Thus, comparison to these trials may not be rational. Higher acidification was observed also in previous studies applying the same strains, after the same fermentation time at 25 °C, but in different matrices (i.e. faba bean and millet) [37, 38].

Acidity induced by LAB may influence on various factors. Decreased pH may bring beneficial changes in the matrix, for instance by activating the endogenous enzymes, which in turn, e.g. enhance the bioavailability of minerals [42, 43]. Besides, acids synthesized by LAB likely play a role in the inhibition of harmful microorganisms [78]. Acidity may also have an impact on the dextransucrase activity [58]. Optimal pH for *L. mesenteroides* NRRL B-512 dextransucrase activity is reported to be 5-5.2 [59, 79]. Moreover, the best pH of *L. mesenteroides* IBUN 91.2.98 dextransucrase activity was 5.0 [80], whereas *W. cibaria* JAG8 dextransucrase had optimal conditions at pH 5.4 [81]. Notably, these values may not apply for all dextransucrases and all strains. Even though moderately low pH appears to allow the dextransucrase activity, LAB fermentation of cereals may commonly lead to even more acidic conditions, observed for instance during sourdough fermentation [82]. Therefore, for dextran

synthesis, relatively mild acidification may be a desired property of a starter. Organic acids have great influences in food product characteristics, and a good balance of the acidity, in particular in the ratio of lactic and acetic acid produced during fermentation, should be taken into account to avoid impairing the technological and sensorial properties [62, 63, 74]. Eventually, fairly mild acidity obtained in all fermented BSG matrices obtained in the present study may enhance their applicability in diverse food products, but the quantification of lactic and acetic acid in the samples is necessary, and currently undergoing [62, 63, 74].

From the examined fermentation temperatures, the greatest viscosity increase, and thus the highest potential for dextran synthesis, was achieved at 25 °C. Thus, 25 °C was considered more suitable fermentation temperature for this purpose, aiming for high dextran yields. Result is in agreement with previous studies regarding these strains in particular. *L. pseudomesenteroides* DSM20193 has previously showed abundant dextran synthesis at 25 °C in faba bean fermentation [37], and *W. confusa* A16 and 2LABPTO5 in millet fermentation [38].

In the present study, significant viscosity increase was achieved in dextran containing samples, thus the rheological properties of BSG were transformed. *L. pseudomesenteroides* DSM20193 showed similar viscosity values, but lower dextran yield in comparison to examined *W. confusa* strains. This might be explained by different structures of the dextran produced and as a consequence, different interactions with the carbohydrate and protein components of BSG. In fact, the dextran structures of the same strains selected in this study, have been studied. Using MRS agar with sucrose supplementation as a substrate, DSM20193 produced dextran with 5.8 % branching via α-1,3-linkages, and weight average molar mass of ~4.4 10^6 g mol^{-1} , whereas A16 formed dextran with 3 % branching via α-1,3-linkages and weight average molar mass of ~3.3 10^6 g mol^{-1} [32, 38]. Dextran synthesized by 2LABPTO5 was nearly identical to A16, but this data was not published in the study [38]. However, since the substrate properties and cultivation conditions greatly influence on the nature of dextran synthesized by LAB, structures formed in the conditions of this study in BSG might be different from the structures analyzed on MRS [40, 56, 57].

Final cell density of presumptive LAB after fermentation with selected starter strains, at both temperatures, is in the same order of magnitude obtained in previous studies of EPS synthesis in cereal matrices [33, 62, 74, 83]. Furthermore, cell density generally increased quite similarly in EPS- and EPS+ samples. These observations indicate the suitability of BSG used in present study to sufficiently fulfill the nutritional requirements of selected LAB strains

[47, 48, 51].

Besides of presumably supporting the growth of desired starters, high moisture level of BSG, combined with the presence of nutrients, may expose it for microbial spoilage, which has been recognized as a challenge regarding its utilization in food [1, 3, 4, 84]. Filamentous fungi, yeasts and bacteria are often detected from cereal grains, and molds commonly represent the main concern [86]. According to Bianchini and Stratton [86] bacteria belonging to the families *Bacillaceae, Micrococcaceae, Lactobacillaceae,* and *Pseudomonadaceae* are frequently detected, and also enteric bacteria have been encountered in cereal crops [85, 86]. In previous study by Bokulich et al. [87], several mold and yeast species, other fungi, lactic and acetic acid bacteria, *Bacillaceae, Micrococcaceae, Staphylococcaceae* and *Enterobacteriaceae* were detected from brewery surfaces [88]. In particular, preparation surfaces of wort, malt and hot side areas, where BSG is formed, were mostly contaminated with *Enterobacteriaceae, Leuconostocaceae, Candida, Pichia,* and *Rhodotorula* spp., which the authors presumed to mainly originate from the grains used as raw material for mashing [87]. However, due to variation in BSG composition between malt types and methods of different manufacturers, variation in microbial quality of BSG can be expected as well [1, 3, 4]. Notably, mashing appears to lead in a significant reduction of microbial load, likely due to relatively high temperatures (above 70 °C) reached during the process, highlighting the effect of contaminations taking place after mashing and storage conditions on the microbiological quality of BSG [4, 68, 84, 88]. In this study, nonappearance of colony forming units of *Enterobacteriaceae, B. cereus,* yeasts and molds and low density of microbes in fresh BSG matrix thus indicates sufficient storage conditions of the BSG as received by the manufacturer.

Fermentation trials in the present study were performed at temperatures suitable for microbial proliferation. Indeed, the outcome of spontaneous fermentation was highly variable, and *B. cereus* and *Enterobacteriaceae* were occasionally detected. Therefore, potential propagation of unwanted microbes in the substrate underlines the necessity for controlled fermentation with defined, robust starter strains. Due to low intrinsic cell density of presumptive LAB in the matrix, inoculum of 6 $\log_{10}$ cfu g^{-1}, often adopted in other studies, likely allowed the starter to be dominant in the time frame considered, enabling the formation of desired characteristics [37, 38, 49]. Nonappearance of *B. cereus, Enterobacteriaceae* in samples fermented by the starter strains, may indicate unfavorable growth conditions, possibly caused by metabolic activities of LAB, such as acidification [78, 89, 90]. Therefore, selected

started strains appeared potential candidates regarding the microbiological quality of the process. These results are promising considering the future utilization of fermented BSG in food applications. Aiming for food use, more thorough microbial safety assessment including greater diversity of microbial groups, could be considered. Worth noting, bacterial colonies appeared on the media aimed for yeast and mold enumeration, and thus the chloramphenicol supplementation was not successful in the purpose of prevention of bacterial growth in this study, which is presumably due to incorrect application.

During fermentation trials at 25 °C, great majority of sucrose was presumably converted to dextran and fructose, formed in nearly equal amounts in all EPS+ samples. As no intrinsic sucrose was detected, 4 w/w % (of wet weight) sucrose supplementation likely served as a substrate for dextran formation. Since dextransucrase ideally converts sucrose into equivalent amounts of dextran and fructose during fermentation, theoretical maximum yield for each is 2 % w/w [23]. Fermentation by *W. confusa* strains A16 and 2LABPTO5 resulted in high dextran yields near the theoretical amount, whereas *L. pseudomesenteroides* DSM20193 yielded somewhat less. In previous studies, fermentation with A16 resulted in high quantity of dextran (~1.4 % of wet weight) in pearl millet sourdough as well and DSM20193 also synthesized considerable amount in faba bean fermentation (~1.45 % of wet weight), at the same fermentation temperature and time [37, 38]. Thus, dextran yields in BSG fermentation in the present study are somehow comparable to those previously observed, although due to different substrate properties, differences in the outcome can be expected [47-50, 56]. Eventually, these strains appear robust performers in dextran synthesis in diverse matrices, including BSG.

Dextran and fructose quantities were rather well correlated in this study. Similarly as dextran, fructose quantity nearly reached the theoretical maximum in A16 and 2LABPTO5 samples, and in DSM20193 sample it was considerably lower. Fructose is fermented by *Leuconostoc* spp. (except some *L. mesenteroides* subsp. *cremoris* strains), and it can be reduced to mannitol, gaining ATP, which may thus explain the lesser fructose quantity [28, 73]. Generally, *W. confusa* are capable of fructose fermentation [69], but since fructose content after fermentation was near the theoretical maximum, it is likely that these strains mainly preferred glucose, maltose or, some other compound not detected in the present study and possibly liberated during trials, in their energy metabolism. Moreover, part of the supplemented sucrose was perhaps also consumed as a fermentable substrate.

Isomaltooligosaccharides, were formed in all EPS+ samples, induced by maltose

present in raw material, which presumably also affected the dextran yield [57, 61]. Nevertheless, isomaltooligosaccharides have been suggested as promising prebiotics [91]. Clarifying the nature and impacts of formed isomaltooligosaccharides is of interest in future studies.

In conclusion, the rheological properties of BSG were modified via LAB fermentation and dextran synthesis, resulting in more viscous texture, and its applicability in food systems was potentially enhanced. *W. confusa* strains A16 and 2LABPTO5 and *L. pseudomesenteroides* strain DSM20193 appeared promising starters for dextran synthesis in this matrix. BSG in the present study endorsed a sufficient growth, mild acidification and abundant dextran synthesis of the selected starters, matrices therefore appearing as potential functional ingredients for novel cereal-based foods. A more detailed examination of the metabolic interactions during BSG fermentation as well as of the interactions between BSG components, formed dextran and food systems will be the subject of further studies.

Acknowledgements

The project “Funbrew – Biotransformation of brewer’s spent grain: increased functionality for novel food application” (ERA-NET Susfood) is acknowledged for providing the funding for this study. Adjunct professor Rossana Coda is acknowledged for supervising this study and PhD student Yaqin Wang for her help with the chemical analyses.

References

1. Lynch KM, Steffen EJ, Arendt EK. Brewers' spent grain: a review with an emphasis on food and health. *Journal of the Institute of Brewing* 2016 122(4): 553-568. doi: 10.1002/jib.363

2. Mussatto SI, Dragone G, Roberto IC. Brewers' spent grain: generation, characteristics and potential applications. *Journal of Cereal Science* 2006 43(1): 1-14. doi: 10.1016/j.jcs.2005.06.001.

3. Mussatto SI. Brewer's spent grain: a valuable feedstock for industrial applications. *Journal of the Science of Food and Agriculture* 2014 94(7): 1264-1275. doi: 10.1002/jsfa.6486

4. Robertson JA, I'Anson KJ, Treimo J, Faulds CB, Brocklehurst TF, Eijsink VG, Waldron KW. Profiling brewers' spent grain for composition and microbial ecology at the site of production. *LWT-Food Science and Technology* 2010 43(6): 890-896. doi: 10.1016/j.lwt.2010.01.019

5. McCarthy AL, O'Callaghan YC, Piggott CO, FitzGerald RJ, O'Brien NM. Brewers' spent grain; bioactivity of phenolic component, its role in animal nutrition and potential for incorporation in functional foods: a review. *Proceedings of the Nutrition Society* 2013 72(1): 117-125. doi: 10.1017/S0029665112002820

6. Moreira MM, Morais S, Carvalho DO, Barros AA, Delerue-Matos C, Guido LF. Brewer's spent grain from different types of malt: Evaluation of the antioxidant activity and identification of the major phenolic compounds. *Food Research International* 2013 54(1): 382-388. doi: 10.1016/j.foodres.2013.07.023

7. Santos M, Jiménez JJ, Bartolomé B, Gómez-Cordovés C, del Nozal MJ. Variability of brewer’s spent grain within a brewery. *Food Chemistry* 2003 80(1): 17-21. doi: 10.1016/S0308-8146(02)00229-7.

8. Waters DM, Jacob F, Titze J, Arendt EK, Zannini E. Fibre, protein and mineral fortification of wheat bread through milled and fermented brewer’s spent grain enrichment. *European Food Research and Technology* 2012 235(5): 767-778. doi: 10.1007/s00217-012-1805-9

9. Mussatto SI, Roberto IC. Chemical characterization and liberation of pentose sugars from brewer's spent grain. *Journal of Chemical Technology and Biotechnology* 2006 81(3): 268-274. doi: 10.1002/jctb.1374

10. Combest S, Warren C. Perceptions of college students in consuming whole grain foods made with brewers’ spent grain. *Food Science & Nutrition* 2019 7: 225–237. doi: 10.1002/fsn3.872

11. Ktenioudaki A, O’Shea N, Gallagher E. Rheological properties of wheat dough supplemented with functional by-products of food processing: Brewer’s spent grain and apple

pomace. *Journal of Food Engineering* 2013 116(2): 362-368. doi: 10.1016/j.jfoodeng.2012.12.005.

12. Stojceska V, Ainsworth P. The effect of different enzymes on the quality of high-fibre enriched brewer's spent grain breads. *Food Chemistry* 2008 110(4): 865-872. doi: 10.1016/j.foodchem.2008.02.074.

13. Ktenioudaki A, Chaurin V, Reis SF, Gallagher E. Brewer's spent grain as a functional ingredient for breadsticks. *International Journal of Food Science & Technology* 2012 47(8): 1765-1771. doi: 10.1111/j.1365-2621.2012.03032.x

14. Ktenioudaki A, Alvarez-Jubete L, Smyth TJ, Kilcawley K, Rai DK, Gallagher E. Application of bioprocessing techniques (sourdough fermentation and technological aids) for brewer's spent grain breads. *Food Research International* 2015;73:107-116. doi: 10.1016/j.foodres.2015.03.008.

15. Ktenioudaki A, Crofton E, Scannell AGM, Hannon JA, Kilcawley KN, Gallagher E. Sensory properties and aromatic composition of baked snacks containing brewer's spent grain. *Journal of Cereal Science* 2013 57(3): 384-390. doi: 10.1016/j.jcs.2013.01.009

16. Finley JW, Hanamoto MM. Milling and baking properties of dried brewer's spent grains. *Cereal Chemistry* 1980 57(3): 166-168.

17. Stojceska V. Chapter 16 - Dietary Fiber from brewer's spent grain as a functional ingredient in bread making technology. In: Preedy VR, Watson RM, Patel VB, editors. *Flour and Breads and their Fortification in Health and Disease Prevention*. Academic Press 2011: 171-181. doi: 10.1016/B978-0-12-380886-8.10016-9

18. Lynch KM, Coffey A, Arendt EK. Exopolysaccharide producing lactic acid bacteria: Their techno-functional role and potential application in gluten-free bread products. *Food Research International* 2018 110: 52-61. doi: 10.1016/j.foodres.2017.03.012.

19. Lynch KM, Zannini E, Coffey A, Arendt EK. Lactic acid bacteria exopolysaccharides in foods and beverages: isolation, properties, characterization, and health benefits. *Annual Review of Food Science and Technology* 2018 9(1): 155-176.

20. Lacaze G, Wick M, Cappelle S. Emerging fermentation technologies: development of novel sourdoughs. *Food Microbiology* 2007 24(2): 155-160. doi: 10.1016/j.fm.2006.07.015.

21. Park JK, Khan T. Other microbial polysaccharides: pullulan, scleroglucan, elsinan, levan, alternant, dextran. In: Phillips GO, Williams PA, editors. *Handbook of hydrocolloids*, 2nd ed. Woodhead Publishing. *2*009: 592-614.

22. Monsan P, Bozonnet S, Albenne C, Joucla G, Willemot R, Remaud-Siméon M. Homopolysaccharides from lactic acid bacteria. *Internatioal Dairy Journal* 2001 11(9): 675-685. doi: 10.1016/S0958-6946(01)00113-3

23. Kothari D, Das D, Patel S, Goyal A. Dextran and food application. In: Ramawat K., Mérillon JM, editors. *Polysaccharides: bioactivity and biotechnology*. Springer 2015: 735-752. doi: 10.1007/978-3-319-16298-0_66

24. Jeanes A, Haynes WC, Wilham CA, Rankin JC, Melvin EH, Austin MJ, Cluskey JE, Fisher BE, Tsuchiya HM, Rist CE. Characterization and classification of dextrans from ninety-six strains of bacteria 1b. *Journal of the American Chemical Society* 1954 76(20): 5041-5052. doi: 10.1021/ja01649a011

25. Werning ML, Notararigo S, Nácher-Vázquez M, Fernández de Palencia P, Aznar R, López P. Biosynthesis, purification and biotechnological use of exopolysaccharides produced by lactic acid bacteria. In: El-Samragy Y. *Food Additive*. InTechOpen 2012: 83-114. doi: 10.5772/32412

26. Badel S, Bernardi T, Michaud P. New perspectives for Lactobacilli exopolysaccharides. *Biotechnology Advances* 2011 29(1): 54-66. doi: 10.1016/j.biotechadv.2010.08.011

27. Leemhuis H, Pijning T, Dobruchowska JM, van Leeuwen SS, Kralj S, Dijkstra BW, Dijkhuizen L. Glucansucrases: three-dimensional structures, reactions, mechanism, α-glucan analysis and their implications in biotechnology and food applications. *Journal of Biotechnology* 2013 163(2): 250-272. doi: 10.1016/j.jbiotec.2012.06.037.

28. Nieminen T, Säde E, Endo A, Johansson P, Björkroth K. The Family *Leuconostocaceae*. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F, editors. *The Prokaryotes*. Springer 2014: 215-240. doi: 10.1007/978-3-642-30120-9_208

29. Naessens M, Cerdobbel A, Soetaert W, Vandamme EJ. *Leuconostoc* dextransucrase and dextran: production, properties and applications. *Journal of Chemical Technology and Biotechnology* 2005 80(8): 845-860. doi: 10.1002/jctb.1322

30. Zhang Y, Guo L, Xu D, Li D, Yang N, Chen F, et al. Effects of dextran with different molecular weights on the quality of wheat sourdough breads. *Food Chemistry* 2018 256: 373-379. doi: 10.1016/j.foodchem.2018.02.146

31. Rühmkorf C, Rübsam H, Becker T, Bork C, Voiges K, Mischnick P, et al. Effect of structurally different microbial homoexopolysaccharides on the quality of gluten-free bread. *European Food Research and Technology* 2012 235(1): 139-146. doi: 10.1007/s00217-012-1746-3

32. Xu Y, Pitkänen L, Maina NH, Coda R, Katina K, Tenkanen M. Interactions between fava bean protein and dextrans produced by *Leuconostoc pseudomesenteroides* DSM 20193 and *Weissella cibaria* Sj 1b. *Carbohydrate Polymers* 2018 190: 315-323. doi: 10.1016/j.carbpol.2018.02.082

33. Katina K, Maina NH, Juvonen R, Flander L, Johansson L, Virkki L, et al. In situ production and analysis of *Weissella confusa* dextran in wheat sourdough. *Food Microbiology* 2009 26(7): 734-743. doi: 10.1016/j.fm.2009.07.008.

34. Zannini E, Waters DM, Arendt EK. The application of dextran compared to other hydrocolloids as a novel food ingredient to compensate for low protein in biscuit and wholemeal wheat flour. *European Food Research and Technology* 2014 238(5): 763-771. doi: 10.1007/s00217-014-2161-8

35. Kajala I, Shi Q, Nyyssölä A, Maina NH, Hou Y, Katina K, Tenkanen M, Juvonen R. Cloning and characterization of a *Weissella confusa* dextransucrase and its application in high fibre baking. *PloS one* 2015 10(1): e0116418. doi: 10.1371/journal.pone.011641

36. Galle S, Schwab C, Dal Bello F, Coffey A, Gänzle MG, Arendt EK. Influence of *in-situ* synthesized exopolysaccharides on the quality of gluten-free sorghum sourdough bread. *International Journal of Food Microbiology* 2012 155(3): 105-112. doi: 10.1016/j.ijfoodmicro.2012.01.009

37. Wang Y, Sorvali P, Laitila A, Maina NH, Coda R, Katina K. Dextran produced *in situ* as a tool to improve the quality of wheat-faba bean composite bread. *Food Hydrocolloids* 2018 84: 396-405. doi: 10.1016/j.foodhyd.2018.05.042

38. Wang Y, Compaoré-Sérémé D, Sawadogo-Lingani H, Coda R, Katina K, Maina NH. Influence of dextran synthesized *in situ* on the rheological, technological and nutritional properties of whole grain pearl millet bread. *Food Chemistry* 2019 285: 221-230. doi: 10.1016/j.foodchem.2019.01.126.

39. Tieking M, Gänzle MG. Exopolysaccharides from cereal-associated lactobacilli. *Trends in Food Science & Technology* 2005 16(1): 79-84. doi: 10.1016/j.tifs.2004.02.015

40. Galle S, Arendt EK. Exopolysaccharides from sourdough lactic acid bacteria. *Critical Reviews in Food Science and Nutrition* 2014 54(7): 891-901. doi: 10.1080/10408398.2011.617474

41. Waldherr FW, Vogel RF. Commercial exploitation of homo-exopolysaccharides in non-dairy food. In: Ullrich, M, editor. *Bacterial polysaccharides: Current innovations and future trends*. Caister Academic Press 2009: 314-329.

42. Poutanen K, Flander L, Katina K. Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology* 2009 26(7): 693-699. doi: 10.1016/j.fm.2009.07.011

43. Reale A, Konietzny U, Coppola R, Sorrentino E, Greiner R. The importance of lactic acid bacteria for phytate degradation during cereal dough fermentation. *Journal of Agricultural and Food Chemistry* 2007 55(8): 2993-2997. doi: 10.1021/jf063507n

44. Marko A, Rakická M, Mikušová L, Valík Ľ, Šturdík E. Lactic acid fermentation of cereal substrates in nutritional perspective. *International Journal of Research in Chemistry and Environment* 2014 4(4): 80-92.

45. Lopez HW, Ouvry A, Bervas E, Guy C, Messager A, Demigne C, et al. Strains of lactic acid bacteria isolated from sour doughs degrade phytic acid and improve calcium and magnesium solubility from whole wheat flour. *Journal of Agricultural and Food Chemistry* 2000 48(6): 2281-2285. doi: 10.1021/jf000061g

46. Coda R, Rizzello CG, Pinto D, Gobbetti M. Selected lactic acid bacteria synthesize antioxidant peptides during sourdough fermentation of cereal flours. *Applied and Environmental Microbiology* 2012 78(4):1087-1096. doi: 10.1128/AEM.06837-11.

47. Hayek SA, Ibrahim SA. Current limitations and challenges with lactic acid bacteria: a review. *Food and Nutrition Sciences* 2013 4(11A): 73-87. doi: 10.4236/fns.2013.411A010.

48. Narvhus JA, Axelsson L. Lactic acid bacteria. In: Caballero B, editor. *Encyclopedia of Food Sciences and Nutrition* 2nd ed. Academic Press 2003: 3465-3472. doi: 10.1016/B0-12-227055-X/00673-8

49. Kajala I, Mäkelä J, Coda R, Shukla S, Shi Q, Maina NH, Juvonen R, Ekholm P, Goyal A, Tenkanen M, Katina K. Rye bran as fermentation matrix boosts in situ dextran production by *Weissella confusa* compared to wheat bran. *Applied Microbiology and Biotechnology* 2016 100(8): 3499-3510. doi: 10.1007/s00253-015-7189-6

50. Wolter A, Hager AS, Zannini E, Galle S, Gänzle MG, Waters DM, et al. Evaluation of exopolysaccharide producing *Weissella cibaria* MG1 strain for the production of sourdough from various flours. *Food Microbiology* 2014 37: 44-50. doi: 10.1016/j.fm.2013.06.009

51. Liptáková D, Matejčeková Z, Ľubomír V. Lactic acid bacteria and fermentation of cereals and pseudocereals. In: Jozala AF, editor. *Fermentation Processes*. IntechOpen 2017: 223-254. doi: 10.5772/65459

52. Hammes WP, Brandt MJ, Francis KL, Rosenheim J, Seitter MFH, Vogelmann SA. Microbial ecology of cereal fermentations. *Trends in Food Science & Technology* 2005;16(1):4-11. doi: 10.1016/j.tifs.2004.02.010

53. Hammes WP, Gänzle MG. Sourdough breads and related products. In: Wood BJB, editor. *Microbiology of Fermented Foods Boston* , 2nd ed. Springer 1998: 199-216. https://doi.org/10.1007/978-1-4613-0309-1_8

54. Xiros C, Christakopoulos P. Biotechnological potential of brewers spent grain and its recent applications. *Waste and Biomass Valorization* 2012 3(2): 213-232. doi: 10.1007/s12649-012-9108-8

55. Novik GI, Wawrzynczyk J, Norrlow O, Szwajcer-Dey E. Fractions of barley spent grain as media for growth of probiotic bacteria. *Microbiology* 2007 76(6): 804. doi: 10.1134/S0026261707060227

56. Kim D, Robyt JF, Lee S, Lee J, Kim Y. Dextran molecular size and degree of branching as a function of sucrose concentration, pH, and temperature of reaction of *Leuconostoc mesenteroides* B-512FMCM dextransucrase. *Carbohydrate Research* 2003 338(11): 1183-1189. doi: 10.1016/S0008-6215(03)00148-4

57. Korakli M, Vogel RF. Structure/function relationship of homopolysaccharide producing glycansucrases and therapeutic potential of their synthesised glycans. *Applied Microbiology and Biotechnology* 2006 71(6): 790-803. doi: 10.1007%2Fs00253-006-0469-4

58. Santos M, Teixeira J, Rodrigues A. Production of dextransucrase, dextran and fructose from sucrose using *Leuconostoc mesenteroides* NRRL B512(f). *Biochemical Engineering Journal* 2000 4(3): 177-188. doi: 10.1016/S1369-703X(99)00047-9

59. Tsuchiya HM, Hellman NN, Koepsell HJ, Corman J, Stringer CS, Rogovin SP, Bogard MO, Bryant G, Feger VH, Hoffman CA, Senti FR, Jackson RW. Factors affecting molecular weight of enzymatically synthesized dextran[2]. *Journal of the American Chemical Society* 1955 77(9): 2412-2419. doi: 10.1021/ja01614a016

60. Tsuchiya HM, Koepsell HJ, Corman J, Bryant G, Bogard MO, Feger VH, Jackson RW. The effect of certain cultural factors on production of dextransucrase by *Leuconostoc mesenteroides*. *Journal of Bacteriology* 1952 64(4): 521.

61. Robyt JF, Walseth TF. The mechanism of acceptor reactions of *Leuconostoc mesenteroides* B-512F dextransucrase. *Carbohydrate Research* 1978 61(1): 433-445. doi: 10.1016/S0008-6215(00)84503-6.

62. Kaditzky S, Vogel RF. Optimization of exopolysaccharide yields in sourdoughs fermented by lactobacilli. *European Food Research and Technology* 2008 228(2): 291. doi: 10.1007/s00217-008-0934-7.

63. Kaditzky S, Seitter M, Hertel C, Vogel RF. Performance of *Lactobacillus sanfranciscensis* TMW 1.392 and its levansucrase deletion mutant in wheat dough and comparison of their impact on bread quality. *European Food Research and Technology* 2008 227(2): 433-442. doi: 10.1007/s00217-007-0738-1

64. Katina K, Salmenkallio-Marttila M, Partanen R, Forssell P, Autio K. Effects of sourdough and enzymes on staling of high-fibre wheat bread. *LWT - Food Science and Technology* 2006 39(5): 479-491. doi: 10.1016/j.lwt.2005.03.013.

65. Xu Y, Wang Y, Coda R, Säde E, Tuomainen P, Tenkanen M, Katina K. In situ synthesis of exopolysaccharides by *Leuconostoc* spp. and *Weissella* spp. and their rheological impacts in fava bean flour. *International Journal of Food Microbiology* 2017;248:63-71. doi: 10.1016/j.ijfoodmicro.2017.02.012.

66. Yeast Extract–Peptone–Dextrose (YPD) Medium (Liquid or Solid). *Cold Spring Harbor Protocols.* Cold Spring Harbor Laboratory Press 2017(8): pdb.rec090563. doi:10.1101/pdb.rec090563

67. Gänzle MG. Enzymatic and bacterial conversions during sourdough fermentation. *Food Microbiology* 2014 37: 2-10. doi: 10.1016/j.fm.2013.04.007.

68. Robertson JA, I'Anson KJA, Brocklehurst TF, Faulds CB, Waldron KW. Effect of storage conditions on the microbial ecology and biochemical stability of cell wall components in brewers' spent grain. *Journal of Agricultural and Food Chemistry* 2010 58(12): 7266-7272. doi: 10.1021/jf1001099

69. Fusco V, Quero GM, Cho G, Kabisch J, Meske D, Neve H, Bockelmann W, Franz C. The genus *Weissella*: taxonomy, ecology and biotechnological potential. *Frontiers in microbiology* 2015 6: 155. doi: 10.3389/fmicb.2015.00155

70. Collins MD, Samelis J, Metaxopoulos J, Wallbanks S. Taxonomic studies on some leuconostoc-like organisms from fermented sausages: description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species. *The Journal of Applied Bacteriology* 1993 75(6): 595-603. doi: 10.1111/j.1365-2672.1993.tb01600.x

71. De Vuyst L, Degeest B. Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiology Reviews* 1999 23(2): 153-177. doi: 10.1111/j.1574-6976.1999.tb00395.x

72. Fessard A, Remize F. Why are *Weissella* spp. not used as commercial starter cultures for food fermentation? *Fermentation* 2017 3(3). doi: 10.3390/fermentation3030038

73. Wisselink HW, Weusthuis RA, Eggink G, Hugenholtz J, Grobben GJ. Mannitol production by lactic acid bacteria: a review. *International Dairy Journal* 2002 12(2): 151-161. doi: 10.1016/S0958-6946(01)00153-4

74. Galle S, Schwab C, Arendt E, Gänzle M. Exopolysaccharide-forming *Weissella* strains as starter cultures for sorghum and wheat sourdoughs. *Journal of Agricultural and Food Chemistry* 2010 58(9): 5834-5841. doi: 10.1021/jf1002683.

75. Schwab C. Formation of oligosaccharides and polysaccharides by *Lactobacillus reuteri* LTH5448 and *Weissella cibaria* 10M in sorghum sourdoughs. *Cereal Chemistry* 2008 85(5): 679-684. doi: 10.1094/CCHEM-85-5-0679

76. Zannini E, Mauch A, Galle S, Gänzle M, Coffey A, Arendt EK, Taylor JP, Waters DM. Barley malt wort fermentation by exopolysaccharide-forming *Weissella cibaria* MG1 for the production of a novel beverage. *Journal of Applied Microbiology* 2013 115(6): 1379-1387. doi: 10.1111/jam.12329

77. Gobbetti M, Gänzle M. Physiology and biochemistry of lactic acid bacteria. In: Gobbetti M, Gänzle M, editors. *Handbook on sourdough biotechnology*. Springer Science & Business Media 2013: 183-216. doi: 10.1007/978-1-4614-5425-0_7

78. Kieran, J, Dalmasso M, Zentek J, Mader A, Bruggeman G, Wallace J, De medici D, Fiore A, Prukner-Radivcic E, Lukac M, Axelsson L, Holck A, Ingmer H, Malakauskas M. Microbes versus microbes: control of pathogens in the food chain. *Journal of the Science of Food Agriculture* 2014 94(15): 3079-3089. doi: 10.1002/jsfa.6735

79. Koepsell HJ, Tsuchiya HM. Enzymatic synthesis of dextran. *Journal of Bacteriology* 1952 63(2): 293-295.

80. Florez Guzman GY, Buitrago G, Ospina S. New dextransucrase purification process of the enzyme produced by *Leuconostoc mesenteroides* IBUN 91.2.98 based on binding product and dextranase hydrolysis. *Journal of Biotechnology* 2018 265: 8-14. doi: 10.1016/j.jbiotec.2017.10.019

81. Jagan MR, Goyal A. Purification, optimization of assay, and stability studies of dextransucrase isolated from *Weissella cibaria* JAG8. *Preparative Biochemistry & Biotechnology* 2013 43(4): 329-341. doi: 10.1080/10826068.2012.737400

82. Hansen ÅS. Sourdough bread. In: Arroyo-Lòpez FN, Evranuz EÖ, Hui YH. *Handbook of plant-based fermented food and beverage technology*, 2nd ed. CRC Press 2012: 512-535. doi: 10.1201/b12055

83. Palomba S, Cavella S, Torrieri E, Piccolo A, Mazzei P, Blaiotta G, et al. Polyphasic screening, homopolysaccharide composition, and viscoelastic behavior of wheat sourdough from a *Leuconostoc lactis* and *Lactobacillus curvatus* exopolysaccharide-producing starter culture. *Applied and Environmental Microbiology* 2012 78(8): 2737-2747. doi: 10.1128/AEM.07302-11

84. Kunze W. Technology brewing and malting. 4th ed. VLB 2010.

85. Bianchini A, Stratton J. Spoilage of animal products | Spoilage of plant products: Cereals and cereal flours. In: Batt CA, Tortorello ML, editors. *Encyclopedia of Food Microbiology*, 2nd ed. Oxford: Academic Press 2014: 459-464. doi: 10.1016/B978-0-12-384730-0.00312-8

86. Harris LJ, Shebuski JR, Danyluk MD, Palumbo MS, Beuchat LR. Nuts, seeds, and cereals. In: Doyle M, Buchanan R, editors. *Food Microbiology*. ASM Press 2013: 203-221. doi: 10.1128/9781555818463.ch9

87. Bokulich NA, Bergsveinson J, Ziola B, Mills DA. Mapping microbial ecosystems and spoilage-gene flow in breweries highlights patterns of contamination and resistance. *eLife* 2015 4: e04634. doi: 10.7554/eLife.04634

88. O'Sullivan TF, Walsh Y, O'Mahony A, Fitzgerald GF, Van Sinderen D. A comparative study of malthouse and brewhouse microflora. *Journal of the Institute of Brewing* 1999 105(1): 55-61. doi: 10.1002/j.2050-0416.1999.tb00006.x

89. Røssland E, Langsrud T, Sørhaug T. Influence of controlled lactic fermentation on growth and sporulation of *Bacillus cereus* in milk. *International Journal of Food Microbiology* 2005 103(1): 69-77. doi: 10.1016/j.ijfoodmicro.2004.11.027.

90. Wong H, Chen Y. Effects of lactic acid bacteria and organic acids on growth and germination of *Bacillus cereus*. *Applied and Environmental Microbiology* 1988 54(9): 2179-2184.

91. Patel S, Majumder A, Goyal A. Potentials of Exopolysaccharides from Lactic Acid Bacteria. *Indian Journal of Microbiology* 2012 52(1): 3-12. doi: 10.1007/s12088-011-0148-8

Supplementary material

Table S1. Viscosity formation by screened LAB (lactic acid bacteria) strains. Initial rows refer to the values before fermentation, and fermented rows to the situation after 24 hours fermentation at 25 ℃. EPS- and EPS+ refer to sample matrices prepared without and with sucrose addition, respectively. N = 2-3.

[a-d] Values in the same group with different superscript letters are significantly different ($p < 0.05$), n = 2-3.

[e] Viscosity increase was obtained by subtracting the initial viscosity from viscosity measured after fermentation. Different viscosity values obtained from different batches of BSG were taken into account in this comparison.

	Viscosity (Pa·s) at shear rate 100 s^{-1}		**Viscosity increase [e] (Pa·s) at shear rate 100 s^{-1}**		**Visually assessed viscosity (0-5)**	
	EPS-	EPS+	EPS-	EPS+	EPS-	EPS+
Initial						
Batch 1	0.1 ± 0.0 [a, b]	0.1 ± 0.0 [a]			0	0
Batch 2	0.3 ± 0.1 [a, b, c]	0.2 ± 0.0 [a, b, c]				
Fermented						
LMG7939	0.1 ± 0.0 [a, b]	0.1 ± 0.0 [a]	0.0 ± 0.0 [a, b]	0.0 ± 0.0 [a, b]	0	0
DSM20193	0.3 ± 0.0 [a, b, c]	3.1 ± 0.5 [d]	-0.1 ± 0.0 [a]	3.0 ± 0.5 [c]	0	5
Sj1b	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	0.0 ± 0.0 [a, b]	0.0 ± 0.0 [a, b]	0	0
LMG17699	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	0.0 ± 0.0 [a, b]	0.0 ± 0.0 [a, b]	0	0.5
Sj5-4	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	0.0 ± 0.0 [a, b]	0.0 ± 0.0 [a, b]	0	0
Sj28-1	0.1 ± 0.0 [a]	0.2 ± 0.0 [a, b, c]	0.0 ± 0.0 [a, b]	0.1 ± 0.0 [a, b]	0	1
DSM5577	0.1 ± 0.0 [a]	0.1 ± 0.0 [a, b, c]	0.0 ± 0.0 [a, b]	0.1 ± 0.0 [a, b]	0	0
C2	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	0.0 ± 0.0 [a, b]	0.0 ± 0.0 [a, b]	0	0
5E7	0.4 ± 0.0 [a, b, c]	0.8 ± 0.2 [b, c]	0.1 ± 0.0 [a, b]	0.6 ± 0.2 [b]	0	1.5
A16	0.2 ± 0.0 [a, b, c]	2.5 ± 0.5 [d]	0.0 ± 0.0 [a, b]	2.4 ± 0.5 [c]	0	5
2LABPTO5	0.2 ± 0.0 [a, b, c]	2.7 ± 0.1 [d]	0.1 ± 0.0 [a, b]	2.6 ± 0.1 [c]	0	5
DSM20194	0.1 ± 0.0 [a, b]	0.3 ± 0.0 [a, b, c]	0.0 ± 0.0 [a, b]	0.2 ± 0.0 [a, b]	0	1
I21	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	0.0 ± 0.0 [a, b]	0.0 ± 0.0 [a, b]	0	0
DSM20343	0.1 ± 0.0 [a]	0.2 ± 0.0 [a, b, c]	0.0 ± 0.0 [a, b]	0.1 ± 0.0 [a, b]	0	0.5
4Rwb	0.5 ± 0.1 [a, b, c]	0.3 ± 0.0 [a, b, c]	0.1 ± 0.1 [a, b]	0.1 ± 0.0 [a, b]	0	0
NEY6	0.4 ± 0.1 [a, b, c]	0.8 ± 0.0 [c]	0.0 ± 0.1 [a, b]	0.6 ± 0.0 [b]	0	1.5