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Leuconostoc citreum TR116: *In-situ* production of mannitol in sourdough and its application to reduce sugar in burger buns

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ABSTRACT

A marketing study revealed that commercially available burger buns can contain up to 10% (w/w) of added sugar. In order to reduce sugar and maintaining the product quality at the same time, functional ingredients and alternative sweetening agents have to be incorporated. In this study, the sourdough lactic acid bacteria *Leuconostoc citreum* TR116, selected for its ability to produce high amounts of mannitol, was used to produce wheat sourdough and its biochemical characteristics (cell count, pH, TTA, sugar- and acid profile, as well as mannitol production) were monitored over 48 h. The so produced sourdough was then incorporated, as a functional ingredient, into a sugar reduced burger bun system and the quality characteristics of the dough and the final product were determined. Sourdough incorporation counteract the negative effects of sugar reduction on dough properties and resulted in the same viscoelastic properties (0.423 ± 0.008) and gluten-network-development (PMT: 160 ± 12.6 s; TM: 44.0 ± 2.6 BU) as the full-sugar control dough. Furthermore, the investigation of specific volume, crumb hardness and chewiness revealed no significant differences between sugar reduced sourdough burger buns and its control. It is noteworthy that sourdough contributed to browning reaction resulting in darker crumb and crust colour (−8.2%; −9.6%) and it extended microbial shelf life of the burger buns significantly (+3.5 days). Sensory evaluation showed no significant differences in sweetness and sourness between sugar reduced buns containing sourdough and the full-sugar control. In conclusion, the incorporation of mannitol-rich sourdough fermented by *Leuconostoc citreum* TR116 represents a novel technological approach in the field of sugar reduction and showed high potential as a functional ingredient to ameliorate the losses of important quality parameters. Especially sourdough containing higher amounts of mannitol and lower amounts of lactate improved significantly the dough and burger bun quality.

1. Introduction

The increasing cases of childhood obesity, type two diabetes and cardiovascular disease, as well as the debate about the introduction of sugar tax in some European countries are the main reasons for the growing demand of sugar reduced products. Consumers in Europe eat in average 50 g added sugar per day, which is double the amount recommended by the WHO (Azaïs-Braesco et al., 2017; WHO, 2015). According to a recently published study, in the US sweet bakery products are the second main source of added sugar, after sweetened beverages, whereas 14.7%/11.4%/10.4% of the total added sugar are consumed in form of sweet baked goods by children/adolescents/adults respectively (Bailey et al., 2018). Furthermore, the baking industry in the UK is advised by the government to reduce the total sugar content by 20% by 2020. Hence, companies are urgently looking for a solution, such as the use of functional ingredients or the implementation of novel technologies, in order to adhere to the new government recommendations ensuring, at the same time, good quality product for consumers. A marketing study revealed that commercially available burger buns can contain up to 8 g of added sugar per unit, which can equate to 10% (w/w) of the whole product. Basically, the consumption of one burger bun covers one third of the recommended daily sugar intake.

Reducing sugar in burger buns is challenging, since it influences important quality parameters, such as volume, texture and taste (Sahin et al., 2017). In order to maintain these attributes, functional ingredients need to be incorporated into the system. In a previous study, the effect of different commercially available polyols on the dough and product quality of sugar reduced and no-added sugar burger buns was

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investigated (Sahin et al., 2018), leading to the conclusion that mannitol showed the most promising results regarding sensory properties; however, the incorporation of other functional ingredients, such as softening agents, stabilizer or preservatives would be needed to ensure to match the physiochemical properties of the commercial buns.

In order to combine the positive effect of mannitol, such as a sugar replacer, and the need for functional ingredients, to mimic the rheology properties exerted by sucrose in wheat dough systems, naturally produced mannitol by sourdough lactic acid bacteria (LAB) can be considered as a key to reduce sugar in baked goods. Sourdough studies reveal an improvement of bread quality (Arendt et al., 2007; Axel et al., 2015; Robert et al., 2006) and an enhancement of flavour and aroma (Montanari et al., 2014; Salim-ur-Rehman et al., 2006). Several studies and reviews about mannitol production by LAB in laboratory media are published and revealed different production yields depending on the microbial strain and the fermentation conditions (Carvalheiro et al., 2011; Gobbetti et al., 2005; Otgonbayar et al., 2011; Saha and Racine, 2011; Wisselink et al., 2002). Amongst all mannitol producing LAB, *Leuconostoc* ssp. can achieve a reduction of fructose to mannitol of up to 95% (Otgonbayar et al., 2011). This study investigates the potential of sourdough as a functional ingredient in sugar reduced burger buns representing a novel technological approach. Therefore, the performance of *Leuconostoc citreum* TR116, in wheat sourdough systems, with and without added fructose, was analysed by evaluating its biochemical traits such as microbial growth, pH, total titratable acids (TTA), sugar profile, mannitol production and acid profile over 48 h in 6 h intervals. Furthermore, *Leuconostoc citreum* TR116 sourdoughs were incorporated in a sugar reduced burger bun system and the effects on dough properties and burger bun quality were investigated.

2. Materials and methods

Sourdough fermentation by using the LAB strain *Leuconostoc citreum* TR116 was performed to produce mannitol. Two different sourdough formulations were tested, one with the addition of fructose and one without, and pH, TTA, as well as microbial cell count, sugar-, polyol- and acid-profile were determined every 6 h over a period of 48 h. Furthermore, the sourdoughs with the optimized fermentation time, regarding to polyol yield, were applied in two different concentrations in a sugar reduced burger bun system.

2.1. Raw materials

Sourdough production was carried out by using wheat flour (bakers flour, Odlums Group, Dublin, Ireland), fructose (Sigma-Aldrich, Gillingham, UK), the LAB strain *Leuconostoc citreum* TR116 and sterile tap water. Burger buns were produced using wheat flour (bakers flour, Odlums, Dublin, Ireland), instant active dried baker's yeast *Saccharomyces cerevisiae* (Puratos, Groot-Bijgaarden, Belgium), sucrose (Siúcra, Dublin, Ireland), salt (Glacia British Salt Limited, Cheshire, UK), wheat gluten (Roquette, Lestrem, France), wheat starch (Roquette, Lestrem, France), ascorbic acid (Storefast Solutions, Northfleet, UK) and sunflower oil (Musgrave Wholesale Partners, Dublin, Ireland), Sodium Stearoyl Lactate (SSL) (Danisco Grindstead Co., Copenhagen, Denmark) and tap water (25 °C).

2.2. Leuconostoc citreum TR116, medium and growth conditions

The LAB strain *Leuconostoc citreum* TR116 used for sourdough fermentation trials, has been previously isolated from yellow pea sourdough and belong to the culture collection of the Department of Biological Sciences, Cork Institute of Technology, Ireland. The strain was stored in a 40% glycerol stock at −80 °C and routinely cultivated on deMan-Rogosa-Sharpe (MRS) agar (Sigma-Aldrich, Gillingham, UK), supplemented with 0.05 g/L bromocresol green (Sigma-Aldrich, Gillingham, UK). Incubation was conducted anaerobically at 30 °C for 48 h.

2.3. Sourdough fermentation

Prior to sourdough production, a cell suspension of the strain TR116 was prepared. Therefore, single colonies were pre-inoculated in 10 mL MRS-broth at 30 °C for 24 h, and subcultured (1%) in 10 mL MRS broth at 30 °C for 16 h. Afterwards, cells were harvest by centrifugation (4500 rpm, 15 min, 4 °C) and washed in 10 mL sterile tap water, followed by another centrifugation step (same settings) and resuspension in sterile tap water. Five-hundred grams of sourdough was produced by the addition of the strain TR116 at a density of 7.0 log CFU/g dough with a dough yield (DY) of 200. Two different sourdoughs were produced. Sourdough 1 (SD1) contained 50% wheat flour, 50% sterile tap water and the starter culture, whereas in sourdough 2 (SD2) 10% of wheat flour was replaced by fructose to trigger mannitol production, resulting in a composition of 40% wheat flour, 10% fructose and 50% sterile tap water. Ingredients were mixed using a Kenwood Major Titanium KM 020 mixer (Kenwood, Havant, UK) at speed 1 for one minute, followed by speed 2 for one minute. Sourdough samples were transferred in sterile stomacher bags and airtightly sealed. Analysis of microbial growth, pH, total titratable acidity (TTA) and sugar-, mannitol- and acid profile and quantification were performed every 6 h over a fermentation period of 48 h starting with time point 0. The pH, TTA and microbial growth were determined directly after sample collection, while the determination of sugars, mannitol and acids required freeze-drying before analysis. All fermentations were performed in triplicates.

2.3.1. Microbial growth and acidification of the sourdough

Microbial growth was determined for each sample taken by homogenising 1 g of sourdough sample in 10 mL of sterile ringer solution using a vortexer and the addition of sterile glass beats. Serial dilution was performed and the enumeration of TR116 was carried out by plating on MRS agar supplemented with 0.05 g/L bromocresol green (Sigma-Aldrich, Gillingham, UK) after anaerobic incubation for 48 h at 30 °C. LAB cell count, pH and total titratable acids (TTA) were determined every 6 h, starting with time point 0 and ending with 48 h. The identity of starter cultures was verified by the morphology and metabolites of the strain (Wolter et al., 2014). TTA and pH values of the fermented sourdoughs were measured using standard procedures (Arbeitsgemeinscaft Getreideforschung e. V., 1994). Additionally, the difference in log cfu/g sourdough between the initial cell count and the value reached after 48 h of fermentation (Δlog), the maximum growth rate (μ_{max}), the difference in pH value between time 0 and 48 h (ΔpH), the maximum acidification rate (ν_{max}) as well as the changes in TTA between 0 h and 48 h (ΔTTA) were determined.

2.3.2. Sugar-, mannitol- and acid quantification of the sourdoughs

For the analysis of sugars (glucose, fructose, sucrose and maltose), mannitol, as well as lactic acid and acetic acid, freeze-dried sourdough samples were extracted in Milli-Q water, clarified with 2.5% (w/v) Carrez I and 2.5% (w/v) Carrez II and syringed filtered (pore size of 0.2 μm). The clarification of the extract for acid analysis was performed by the addition of 2.5% (w/v) 70% perchloric acid. The quantification of glucose (1–50 mmol/L), fructose (1–100 mmol/L), sucrose (1–50 mmol/L) and maltose (1–50 mmol/L), as well as mannitol (1–100 mmol/L), lactic acid (1–20 mmol/L) and acetic acid (1–20 mmol/L) was conducted by using an Agilent 1260 high performance liquid chromatography system equipped with a refractive index detector (RID) and an ultra violet-diode array detector (UV/DAD). Standard calibration curves were determined with 5 different concentrations per compound, and measured at the beginning and at the end of a sample set. The concentration of sugars and mannitol was determined over the RID (40 °C) by elution of the extract from a RezexTM RPM-Monosaccharide Pb + column (300 × 7.8 mm, Phenomenex, California, USA) at 80 °C, equipped with a guard column (Carbo-Pb, 4 × 3.0 mm, Phenomenex, California, USA), using MiliQ-water at a flow rate of 0.6 mL/min and an injection volume of 20 μL.

Lactic acid and acetic acid concentrations in the sourdough samples were analysed by using a Hi-Plex H column (300 × 7.7 mm, 8 mm, Agilent, Cork, Ireland) at 65 °C, equipped with a guard column (50 × 7.7 mm, 8 mm, Agilent, Cork, Ireland) and setting the UV/DAD detector to 210 nm. 0.005 M sulphuric acid was applied as an eluent with a flow rate of 0.5 mL/min. The injection volume of the sample was 20 µL. The extraction was conducted twice for each sample. The identified concentration was given in g/100 g based on dry matter of the freeze-dried sourdough by considering its moisture content (results of moisture content not shown) and the average of 6 values (2 extractions for 3 batches) was determined. Additionally, the fermentation quotient (lactic acid/acetic acid) was calculated.

2.4. Sourdough application in sugar reduced burger buns

Two different sourdoughs, SD1 and SD2, were incorporated in a sugar reduced burger bun system in order to investigate the effect of naturally *in-situ* produced mannitol on the quality of burger buns and their dough properties. Each sourdough was added in two different concentrations, 5% and 10% based on flour.

2.4.1. Dough properties

Several dough analyses, such as gas production during fermentation, changes in the proportion of viscous and elastic properties, extensibility of the dough, the impact of sourdough on the gluten network formation and changes in pasting properties, were performed.

2.4.1.1. Burger bun dough preparation. Sourdoughs were applied in the burger bun recipe containing 5% (w/w) added sucrose. The recipes of all formulations are shown in Table 1. All doughs were produced as previously described by Sahin et al. (2017). Flour and water was replaced by 5% (w/w) or 10% (w/w) sourdough respectively.

2.4.1.2. Dough development and gaseous release during proofing. The determination of the fermentation quality was conducted by using a Rheofermentometer (Chopin, Villeneuve-la-Garenne Cedex, France). 300 g of burger bun dough was placed into a fermentation chamber, a cylindrical weight of 1500 g was put on top of the dough and fermentation was performed at 30 °C for 180 min. The fermentation quality was investigated by the evaluation of several parameters such as, the volume of CO_2 production (V_{tot}), the maximum height of dough (Hm), the maximum height of gaseous release (Hm') and the time required to achieve Hm (T1).

2.4.1.3. Viscoelastic properties of the burger bun dough. A Rheometer Physica MCR 301 (Anton Paar GmbH, Ostfildern, Germany) was used in order to investigate the changes in viscous and elastic proportions of burger bun dough containing different sourdoughs, as well as various sourdough concentrations. For the determination of the viscoelastic properties, the addition of yeast to the burger bun doughs were omitted (Lynch et al., 2009). An oscillating mode was used in combination with parallel plate geometry (50 mm diameter), serrated to prevent slippage. The temperature of the lower plate was set to 30 °C and first an amplitude sweep was performed, as described by Hager et al. (2011) to determine the linear viscoelastic region (data not shown) and to set the target strain for the frequency sweep. Frequency sweep was performed as described by Sahin et al. (2017) using a constant target strain of 0.01% and a frequency range from 100 to 0.1 Hz. The damping factor as an expression of the proportion of viscous and elastic parts in the burger bun dough system was evaluated.

2.4.1.4. Extensibility and resistance to extension. The extensibility and the resistance to extension were determined by using the Extensograph (Brabender, Duisburg, Germany). 150 g dough was moulded and placed in the proofing chamber at 30 °C for 60 min, followed by the measurement and the evaluation of the extensibility and the resistance of extension.

2.4.1.5. Gluten network formation. For the investigation of the impact of sourdough on the gluten network formation GlutoPeak (Brabender, Duisburg, Germany) was used. Therefore, a standard ratio of 50/50 (solid/liquid) of the recipe was weight in, considering the dough yield (200) of the sourdough (50% solid, 50% liquid). The chamber temperature was set to 36 °C and torque was recorded over a period of 600 s. The torque maximum (TM) and the peak maximum time (PMT) were evaluated.

2.4.1.6. Pasting properties. Starch pasting properties were analysed using Rapid Visco Analyser (RVA) (Newport Scientific, Warriewood, Australia). Samples containing 3 g of solids and 25 g of liquids, considering the sourdough as 50% liquid and 50% solid, were prepared. Therefore, 3 g solid ingredients (on a basis of 14% moisture in total) of the recipe were weight into a RVA aluminium sample canister, and the liquid ingredients sunflower oil and 50% sourdough part were added, followed by the inclusion of distilled water up to a total volume of 25 g liquids. The test was conducted as described by Horstmann et al. (2017).

2.4.2. Burger bun quality

The quality of sugar reduced burger buns containing sourdough compared to 10% added sugar burger bun (full-sugar control) and a 5% added sugar burger bun without sourdough addition (reduced-sugar control) was investigated by the evaluation of several properties, such as specific volume, texture, crumb (ultra-) structure, colour, water

Table 1
Recipes of control burger buns and buns with sourdough incorporation in two different concentrations (5% and 10%). Sourdough 1 represents the sourdough with very low amounts of mannitol, whereas sourdough 2 contains mannitol produced during fermentation.

	Full-sugar control	Sugar reduced control	5% Sourdough (1 or 2)	10% Sourdough (1 or 2)
Wheat flour	100	100	95	90
Wheat starch	–	9.4	9.4	9.4
Sourdough (solid part)	–	–	5	10
Sourdough (liquid part)	–	–	5	10
Water	55	62.2	57.2	52.2
Sugar	18.8	9.4	9.4	9.4
Salt	1.7	1.7	1.7	1.7
Sunflower oil	6.7	6.7	6.7	6.7
Baker's yeast	2.0	2.0	2.0	2.0
Wheat gluten	3.2	3.2	3.2	3.2
SSL	0.5	0.5	0.5	0.5
Ascorbic acid	0.1	0.1	0.1	0.1

activity and microbial shelf as well as sensory characteristics. Three burger buns per batch were analysed 1 h after baking. Additionally, texture profile analysis (TPA) was carried out after 24 h, 48 h and 120 h to determine the staling rate.

2.4.2.1. Burger bun preparation. Burger bun dough was scaled to 80 g pieces, moulded, placed on a burger bun tray and baked as previously described by (Sahin et al., 2017). After baking, the buns cooled down on a rack for one hour at room temperature and packed in plastic bags.

2.4.2.2. Specific volume. The determination of the specific volume [mL/g] of the burger buns was conducted by using a 3D laser scan using a VolScan Profiler 300 (Stable Micro Systems, Godalming, UK).

2.4.2.3. Crumb structure and ultrastructure. The crumb structure of the buns was determined using imaging analysis with the C-cell Bread Imaging System (Calibre Control International Ltd., Warrington, UK). Samples were prepared by cutting the bottom and the top of the burger buns to a resulting height of 35 mm using a bread slicer. The area of cells [%] was evaluated. The effect of sourdoughs on the ultrastructure of the crumb was investigated by using scanning electron microscopy (SEM). Burger buns were prepared as described by Sahin et al. (2018), coated with a layer of 25 mm of sputtered palladium-gold and observed using SEM with a working distance of 8 mm. Micrographed were taken at an accelerating voltage of 5 kV and using SEM Control User Interface software, Version 5.21 (JEOL Technics Ltd., Tokyo, Japan).

2.4.2.4. Crumb texture. TA-XT2i texture analyser (Stable Micro Systems, Godalming, UK) was used to investigate the effect of sourdough on texture properties of sugar reduced burger buns. Therefore, the system was equipped with a 25 kg load cell and a cylindrical probe (35 mm) was chosen. Burger buns were prepared using the same manner for crumb structure. The test was performed with a test speed of 5 mm/s, a post-test speed of 10.0 mm/s, a force of 0.05 N and a 5 s waiting time between first and second compression. Burger buns were analysed after 1 h, 24 h, 48 h and 120 h, and the crumb hardness, cohesiveness, springiness, chewiness and resilience were evaluated and the staling rate was calculated using the following formula:

$$\text{Rate of Staling} = (\text{Crumb Hardness 120 h} - \text{Crumb Hardness 2 h}) / \text{Crumb Hardness 2 h}$$

2.4.2.5. Crumb and crust colour. The colour of the burger buns was determined using a hand held colorimeter (Minolta CR-331, Konica Minolta Holdings Inc., Osaka, Japan). The L*-value of the crumb and crust were evaluated.

2.4.2.6. Water activity and microbial shelf life. The determination of the water activity of the crumb was performed by using a water activity meter (HygroLab, Rotronic, Bassersdorf, Switzerland). The influence of sourdough on the shelf life of sugar reduced burger buns was evaluated by using the mould environmental challenge method indicated by Dal Bello et al. (2007). Sahin et al. (2017) described the preparation of the buns for microbial shelf life.

2.4.2.7. Sensory evaluation. The impact of sourdough on the sensory properties of sugar reduced burger buns was investigated by performing an intensity evaluation. A trained panel of 10 people (5 female and 5 male, age: 24–32) was chosen to determine the intensity in crumb hardness (bite), aroma, flavour, sourness and sweetness of the buns. The training of the chosen panel took place 6 h weekly over a period of 6 months prior the evaluation. The definition of the descriptors and the procedure of the training are described by Sahin et al. (2018). The training as well as the sensory evaluation were conducted in a sensory panel room at 21 ± 1 °C. Sensory evaluation was performed in a duplicate.

2.4.3. Statistical analysis

All analyses were performed in triplicates. A variance analysis (one-way ANOVA, $p \leq 0.05$, Tukey test) was performed using Minitab 17. Furthermore, correlation analysis was conducted using Microsoft Excel 2016.

3. Results

In order to investigate the effect of sourdough as a potential sugar replacer in sugar reduced burger buns, the characterisation of two sourdoughs was performed, followed by the application and the determination of several dough properties and product quality parameters.

3.1. Sourdough fermentation

Two different sourdoughs were fermented for 48 h. Sampling took place every 6 h and microbial growth as well as acidification (pH and TTA) were determined. Furthermore, sourdough samples were freeze-dried for the quantification of sugars and metabolites.

In both sourdoughs a cell density of 7 log cfu/g sourdough was determined before fermentation (time point 0), which complied with the inoculation. An increase of cell density in the first 6 h of fermentation (+1.45 ± 0.43 log cfu/g in SD1 and +1.82 ± 0.11 log cfu/g in SD2) was observed, followed by a further growth in SD1 up to 9.40 ± 0.21 log cfu/g and up to 9.21 ± 0.09 log cfu/g in SD2. After 12 h of fermentation a stationary phase of growth occurred, which lasted until 24 h in SD1 and until 36 h in SD2, followed by the decline phase of growth. SD2 showed a significant higher Δlog (2.05 ± 0.08) than SD1 (1.68 ± 0.04) due to a more pronounced death phase in SD1 (−1.80 log cfu/g) resulting in a significant lower cell count compared to SD2 after 48 h. However, the maximum growth rate μ_{max} during the exponential phase of growth of both sourdoughs did not differ significantly from each other (SD1: 0.66 ± 0.07; SD2: 0.72 ± 0.03). The acidification of the sourdoughs is represented by the pH and TTA. Both sourdoughs had a starting pH value of 5.5 and also demonstrated the same end value of 4.16. Furthermore, the maximum acidification rate was 0.14 ± 0.01 pH/h in both sourdoughs and hence did not differ significantly from each other. Before fermentation the TTA equal in both sourdoughs. After fermentation the value increased by 16.80 ± 1.44 mL 0.1 M NaOH in SD2, whereas the increase in TTA in SD1 was significantly lower (+9.53 ± 0.64 mL 0.1 M NaOH). This difference is reflected by the higher ΔTTA in SD2.

The sugar and acid profiles during fermentation are demonstrated in Table 2.

The same amount of sucrose was present in both sourdoughs at the beginning of the fermentation (0 h), whereas after 6 h of fermentation no sucrose was detected anymore. Furthermore, the concentration of maltose in both sourdoughs did not differ at time point 0. During fermentation the amounts of maltose decreased after 6 h, followed by an increase, resulting in a final maltose concentration of 4.95 ± 0.34 g/100 g dry matter (DM) in SD1 and 2.62 ± 0.08 g/100 g DM in SD2. The glucose quantities in both sourdoughs were very low at the beginning of fermentation. However, the amount of glucose increased after 6 h. During fermentation the amounts of glucose increased and decreased slightly over time, resulting in final concentrations of 1.69 ± 0.11 g/100 g DM in SD1 and 1.52 ± 0.22 g/100 g DM in SD2 after 48 h. In both sourdoughs the amounts of fructose differed in starting concentration (0.16 ± 0.04 g/100 g DM in SD1, 11.54 ± 0.25 g/100 g DM in SD2). Nevertheless, fructose quantities decreased over time resulting in a final concentration lower than 0.5 g/100 g DM in both sourdoughs. Mannitol was produced in both sourdoughs. The production of this polyol stopped after 18 h in SD1,

Table 2
Quantities of sugars (sucrose, maltose, glucose, fructose), mannitol and acids (lactic acid and acetic acid) in sourdough 1 (SD1) and sourdough 2 (SD2) in gram per 100 g sourdough based on dry matter (DM) during fermentation. The fermentation quotient is the quotient of the amounts of lactic acid and acetic acid. TTA is presented as mL 0.1 M NaOH, and pH values of sourdough is shown. Each value is given as the average value ± standard deviation, whereas absent molecules are marked as "not detected" (n.d.). Values in one column followed by the same lower case letter are not significantly different ($P < 0.05$).

Fermentation time [h]		Sucrose [g/100gDM]	Maltose [g/100gDM]	Glucose [g/100gDM]	Fructose [g/100gDM]	Mannitol [g/100gDM]	Lactic acid [g/100gDM]	Acetic acid [g/100gDM]	Fermentation quotient	pH	TTA
0	SD1	0.32 ± 0.06 (a)	2.51 ± 0.36 (fg)	0.33 ± 0.08 (j)	< 0.19 (ij)	0.20 ± 0.03 (f)	< 0.10 (i)	n.d.	–	5.50 ± 0.06 (a)	2.82 ± 0.21 (j)
	SD2	0.21 ± 0.16 (b)	2.24 ± 0.62 (fghi)	< 0.37 (j)	11.54 ± 0.25 (a)	< 0.36 (ef)	< 0.09 (i)	< 0.06 (g)	–	5.54 ± 0.16 (a)	2.78 ± 0.13 (j)
6	SD1	n.d.	2.77 ± 0.25 (defg)	0.71 ± 0.04 (i)	< 0.19 (j)	0.55 ± 0.11 (ef)	0.80 ± 0.21 (g)	n.d.	–	5.27 ± 0.06 (b)	4.85 ± 0.16 (i)
	SD2	n.d.	2.21 ± 0.16 (ghi)	1.14 ± 0.04 (de)	10.31 ± 0.43 (b)	0.82 ± 0.12 (ef)	0.39 ± 0.06 (h)	0.09 ± 0.02 (f)	4.33	5.36 ± 0.04 (b)	4.58 ± 0.48 (i)
12	SD1	n.d.	2.49 ± 0.19 (fg)	0.72 ± 0.05 (hi)	< 0.19 (j)	0.73 ± 0.04 (ef)	2.12 ± 0.07 (c)	0.10 ± 0.02 (f)	21.20	4.44 ± 0.10 (cd)	9.25 ± 0.89 (h)
	SD2	n.d.	1.67 ± 0.28 (i)	0.79 ± 0.01 (hi)	4.77 ± 0.23 (c)	3.44 ± 0.38 (d)	1.35 ± 0.02 (f)	0.26 ± 0.03 (e)	5.19	4.50 ± 0.05 (c)	10.80 ± 0.73 (g)
18	SD1	n.d.	3.21 ± 0.28 (de)	1.03 ± 0.01 (efg)	< 0.19 (j)	0.83 ± 0.03 (ef)	2.82 ± 0.14 (b)	0.11 ± 0.02 (f)	25.64	4.24 ± 0.03 (ef)	10.97 ± 0.40 (g)
	SD2	n.d.	1.81 ± 0.30 (hi)	0.87 ± 0.02 (ghi)	2.03 ± 0.07 (d)	6.14 ± 0.75 (c)	1.39 ± 0.07 (ef)	0.36 ± 0.01 (d)	3.86	4.33 ± 0.02 (de)	12.73 ± 1.07 (e)
24	SD1	n.d.	4.55 ± 0.17 (ab)	0.92 ± 0.03 (fgh)	< 0.19 (j)	0.90 ± 0.06 (e)	2.83 ± 0.13 (b)	0.10 ± 0.01 (f)	28.30	4.18 ± 0.03 (f)	11.98 ± 0.16 (fg)
	SD2	n.d.	2.46 ± 0.15 (fg)	1.12 ± 0.06 (de)	1.43 ± 0.16 (e)	8.41 ± 0.63 (b)	1.32 ± 0.03 (f)	0.44 ± 0.05 (c)	3.00	4.27 ± 0.04 (ef)	15.17 ± 0.43 (d)
30	SD1	n.d.	4.11 ± 0.61 (abc)	1.50 ± 0.14 (abc)	< 0.19 (j)	0.98 ± 0.12 (ef)	2.88 ± 0.12 (ab)	0.10 ± 0.02 (f)	28.80	4.20 ± 0.05 (f)	11.47 ± 0.27 (efg)
	SD2	n.d.	2.86 ± 0.35 (def)	1.30 ± 0.05 (cd)	0.78 ± 0.08 (f)	9.08 ± 0.26 (ab)	1.45 ± 0.04 (ef)	0.46 ± 0.02 (bc)	3.15	4.21 ± 0.04 (ef)	17.00 ± 0.59 (c)
36	SD1	n.d.	4.12 ± 0.33 (b)	1.67 ± 0.09 (a)	< 0.19 (j)	0.94 ± 0.08 (e)	3.05 ± 0.17 (a)	0.11 ± 0.01 (f)	27.73	4.18 ± 0.05 (f)	11.77 ± 0.25 (efg)
	SD2	n.d.	2.40 ± 0.18 (fgh)	1.09 ± 0.03 (ef)	0.40 ± 0.05 (ghi)	9.08 ± 0.18 (ab)	1.58 ± 0.05 (de)	0.52 ± 0.06 (a)	3.04	4.20 ± 0.06 (ef)	17.38 ± 0.46 (bc)
42	SD1	n.d.	4.01 ± 0.42 (b)	1.68 ± 0.24 (a)	< 0.19 (j)	0.92 ± 0.09 (e)	2.89 ± 0.10 (ab)	0.12 ± 0.02 (f)	24.08	4.17 ± 0.06 (f)	11.98 ± 0.54 (efg)
	SD2	n.d.	3.34 ± 0.09 (cd)	1.39 ± 0.04 (bc)	0.50 ± 0.04 (g)	9.69 ± 0.42 (a)	1.64 ± 0.07 (d)	0.49 ± 0.02 (abc)	3.35	4.15 ± 0.03 (f)	18.28 ± 0.44 (b)
48	SD1	n.d.	4.95 ± 0.34 (a)	1.69 ± 0.11 (a)	< 0.19 (j)	0.91 ± 0.01 (e)	2.87 ± 0.08 (ab)	0.13 ± 0.02 (f)	22.08	4.16 ± 0.05 (f)	12.35 ± 0.59 (ef)
	SD2	n.d.	2.62 ± 0.08 (efg)	1.52 ± 0.22 (ab)	0.44 ± 0.06 (gh)	9.63 ± 0.25 (a)	1.70 ± 0.05 (d)	0.52 ± 0.03 (ab)	4.27	4.16 ± 0.05 (f)	19.58 ± 1.31 (a)

whereas in SD2 the highest amount of mannitol was reached after 30 h. SD2 contained the 10-fold amount of mannitol compared to SD1 at the end of fermentation.

Regarding the acids, citric acid, malic acid and succinic acid were detected in small amounts (lower than 1 mmol/L) in both sourdoughs (data not shown), whereas the concentration of lactic acid and acidic increased during fermentation. Lactic acid was detected after 6 h and increased over time, resulting in a final concentration of 2.87 ± 0.08 g/100 g DM in SD1 and 1.70 ± 0.05 g/100 g DM in SD2. Acetic acid occurred after 12 h in SD1 and after 6 h in SD2 and the amount also increased over time. The end concentration of acetic acid in SD2 (0.52 ± 0.03 g/100 g DM) was 4 times higher than in SD1 (0.13 ± 0.02 g/100 g DM). The fermentation quotient showed significant higher values in SD1, which reached between 4.1- and 9.4-fold the amount of SD2 over time (Table 3).

3.2. Sourdough application in sugar reduced burger buns

In order to investigate the potential of both sourdoughs as a tool to reduce added sugar in burger buns, SD1 and SD2 were applied in a 50% (w/w) sugar reduced burger bun formulation (5% (w/w) added sucrose) in two concentrations (5% and 10% of flour and water). Based on the results of the kinetic study above, a fermentation time of 30 h was chosen to achieve the highest mannitol production. The sourdoughs were freshly applied and their effect on dough quality (Table 3) and burger bun properties (Table 4) was evaluated.

3.2.1. Effect of sourdoughs on dough properties

The impact of two different sourdoughs in two different concentrations on a burger bun dough was investigated. Therefore, the dough development and gas production during dough fermentation, the extensibility and the resistance to extension, the effect on gluten network formation, as well as the impact of the sourdoughs on the pasting properties of the burger bun dough system, were evaluated.

3.2.1.1. Dough development and gaseous release during proofing. The application of both sourdoughs, regardless the type and amount, did not affect the maximum dough height Hm significantly, neither did it influence the time required to reach this height (T1), or the volume of CO_2 production (V_{tot}) compared to both controls (Table 3). On the other hand, the incorporation of sourdough revealed a significantly higher Hm' value than the full-sugar control (increase of 15.9–22.8%), but significantly lower Hm' than the sugar reduced control (decrease of 24.7–29%). Moreover, Hm' of the burger bun doughs containing SD1 or SD2 did not differ from each other (between 105.0 ± 2.8 mm and 111.3 ± 2.9 mm).

3.2.1.2. Viscoelastic properties. The viscoelastic properties of burger bun doughs were determined by oscillation and are demonstrated in Table 3. The damping factor of the reduced sugar burger bun control with 5% (w/w) added sucrose (0.365 ± 0.011) differed significantly from the damping factor of the full-sugar control dough with 10% (w/w) added sucrose (0.423 ± 0.008). The incorporation of sourdough caused the same viscoelastic dough properties as the full-sugar control dough regardless the type or amount of sourdough added.

3.2.1.3. Extensibility and resistance to extension. The extensibility and the resistance of extension are main characteristics of doughs and reflect the microstructural properties as well as the consistency of the dough. These parameters were determined to evaluate the influence of sourdough on the strength of the dough (Table 3). No significant differences in extensibility of the doughs with sourdough compared to the controls occurred. On the other hand, a significant increase in resistance of extension by the incorporation of 5% sourdough was determined. The addition of 10% SD1 resulted in the same resistance of extension as the full-sugar control dough.

Table 3
Dough properties of burger buns containing sourdough 1 or sourdough 2 compared with the full-sugar- and sugar reduced burger bun controls. Hm represents the maximum dough height, Hm' is the maximum height of gaseous release, T1 reflects the time needed to reach Hm, V_{tot} is the volume of CO_2 production, PMT illustrates the peak maximum time during the gluten network development and TM is the torque maximum reached. Each value is given as the average value ± standard deviation. Values in one column followed by the same lower case letter are not significantly different ($P < 0.05$).

		Hm [mm]	Hm' [mm]	T1 [min]	V_{tot} [mL]	Damping factor	Extensibility [mm]
Control	5% sucrose	62.3 ± 3.7 (a)	147.8 ± 5.0 (a)	126.9 ± 9.2 (a)	2349.4 ± 25.5 (a)	0.364 ± 0.012 (b)	110.5 ± 3.5 (ab)
	10% sucrose	65.3 ± 4.5 (a)	90.6 ± 0.6 (c)	102.8 ± 0.3 (a)	2004.4 ± 14.2 (a)	0.423 ± 0.008 (a)	115.0 ± 0.0 (ab)
Sourdough 1	5%	62.8 ± 9.0 (a)	105.0 ± 2.8 (b)	161.3 ± 26.5 (a)	2337.0 ± 64.7 (a)	0.408 ± 0.011 (a)	116.0 ± 3.9 (a)
	10%	58.4 ± 8.6 (a)	109.2 ± 9.0 (b)	100.5 ± 28.0 (a)	2517.7 ± 260.6 (a)	0.413 ± 0.016 (a)	104.7 ± 2.4 (b)
Sourdough 2	5%	66.0 ± 5.2 (a)	105.1 ± 4.1 (b)	149.0 ± 27.0 (a)	2305.3 ± 121.0 (a)	0.399 ± 0.014 (ab)	114.2 ± 7.3 (a)
	10%	55.6 ± 5.5 (a)	111.3 ± 2.9 (b)	113.5 ± 16.7 (a)	2313.0 ± 68.6 (a)	0.407 ± 0.011 (a)	114.3 ± 1.6 (a)

	Resistance to extension [BU]	PMT [s]	TM [BU]	Peak viscosity [cP]	Breakdown [cP]	Final viscosity [cP]	Pasting temperature [°C]
Control	475.0 ± 21.2 (bc)	114.8 ± 2.4 (d)	49.8 ± 1.0 (a)	865.3 ± 78.8 (a)	324.0 ± 8.0 (a)	1225.0 ± 73.7 (a)	63.6 ± 0.0 (a)
	382.5 ± 3.5 (d)	160.0 ± 12.6 (c)	44.0 ± 2.6 (b)	677.0 ± 22.5 (cd)	236.7 ± 7.4 (d)	968.3 ± 73.9 (c)	66.7 ± 1.6 (a)
Sourdough 1	605.2 ± 30.5 (a)	158.3 ± 4.1 (c)	40.2 ± 2.4 (b)	727.0 ± 18.1 (b)	271.5 ± 5.8 (b)	1112.3 ± 18.1 (ab)	64.2 ± 1.5 (a)
	428.3 ± 19.4 (cd)	186.0 ± 4.7 (b)	35.2 ± 2.2 (c)	716.5 ± 6.0 (bc)	264.5 ± 5.5 (bc)	1046.0 ± 91.4 (bc)	65.9 ± 1.7 (a)
Sourdough 2	616.7 ± 20.7 (a)	158.8 ± 5.4 (c)	40.3 ± 2.3 (b)	715.4 ± 18.8 (bc)	269.6 ± 12.3 (b)	1079.2 ± 14.8 (bc)	65.0 ± 1.3 (a)
	475.0 ± 25.9 (b)	212.3 ± 15.5 (a)	33.5 ± 2.7 (c)	677.0 ± 8.7 (d)	253.8 ± 6.7 (cd)	1026.8 ± 14.2 (bc)	64.9 ± 1.4 (a)

Table 4
Burger bun properties and sensory characteristics of buns containing sourdough 1 or sourdough 2 compared to 10% added sugar and sugar reduced burger bun controls. TPA stands for “texture profile analyser”. Each value is given as the average value ± standard deviation. Values in one column followed by the same lower case letter are not significantly different ($P < 0.05$).

		Specific volume [mL/g]	Crumb hardness (TPA) [N]	Chewiness	Staling rate	Area of cells [%]	L*-value crust	L*-value crumb
Control	5% sucrose	4.47 ± 0.09 (a)	1.73 ± 0.16 (c)	1.24 ± 0.13 (d)	5.94 ± 1.00 (a)	53.7 ± 0.8 (bc)	60.7 ± 0.9 (b)	78.6 ± 1.3 (a)
	10% sucrose	3.51 ± 0.07 (bcd)	4.39 ± 0.79 (a)	2.68 ± 0.57 (ab)	4.58 ± 0.92 (b)	53.2 ± 0.9 (c)	65.1 ± 3.3 (a)	78.22 ± 2.0 (a)
Sourdough 1	5%	3.73 ± 0.36 (b)	3.04 ± 0.73 (b)	2.05 ± 0.49 (c)	5.07 ± 0.82 (ab)	55.7 ± 1.9 (a)	54.9 ± 2.0 (d)	74.2 ± 3.4 (b)
	10%	3.62 ± 0.24 (bc)	3.23 ± 0.39 (b)	2.32 ± 0.47 (bc)	5.18 ± 1.34 (ab)	55.1 ± 0.8 (ab)	56.8 ± 2.6 (c)	71.9 ± 3.0 (cd)
Sourdough 2	5%	3.40 ± 0.34 (cd)	3.45 ± 0.62 (b)	2.31 ± 0.38 (bc)	5.39 ± 0.58 (ab)	53.4 ± 1.6 (bc)	55.2 ± 2.1 (d)	73.0 ± 2.7 (bc)
	10%	3.27 ± 0.06 (d)	5.24 ± 0.60 (a)	3.29 ± 0.44 (a)	4.42 ± 0.67 (b)	52.5 ± 1.1 (c)	59.4 ± 1.0 (b)	70.69 ± 1.6 (d)

	Water activity	Shelf life [d]	Sweetness	Sourness	Aroma	Flavour	Hardness (bite)
Control	0.937 ± 0.005 (b)	6.5 ± 0.7 (c)	3.67 ± 1.21 (a)	2.67 ± 1.89 (a)	6.67 ± 2.22 (ab)	6.44 ± 1.40 (ab)	4.28 ± 1.87 (a)
	0.914 ± 0.006 (c)	10.0 ± 0.0 (a)	6.00 ± 1.26 (a)	2.17 ± 2.62 (a)	7.28 ± 1.44 (a)	6.78 ± 1.18 (a)	4.50 ± 1.94 (a)
Sourdough 1	0.947 ± 0.003 (a)	7.0 ± 0.0 (c)	4.33 ± 1.63 (a)	1.44 ± 1.36 (a)	4.33 ± 2.19 (b)	4.78 ± 1.30 (ab)	3.83 ± 1.50 (a)
	0.951 ± 0.002 (a)	10.0 ± 0.0 (a)	4.50 ± 1.22 (a)	1.94 ± 1.59 (a)	4.94 ± 1.78 (ab)	4.89 ± 1.71 (ab)	4.06 ± 1.47 (a)
Sourdough 2	0.950 ± 0.007 (a)	7.5 ± 0.7 (bc)	4.50 ± 2.26 (a)	1.78 ± 1.66 (a)	5.00 ± 2.05 (ab)	4.56 ± 1.31 (b)	4.11 ± 1.27 (a)
	0.955 ± 0.006 (a)	9.0 ± 0.0 (ab)	5.00 ± 1.67 (a)	2.39 ± 2.06 (a)	5.39 ± 2.53 (ab)	5.89 ± 1.62 (ab)	4.00 ± 1.00 (a)

3.2.1.4. Gluten network development. The impact of two different types of sourdough in two different concentrations on the gluten network formation was determined by the evaluation of the torque maximum (TM) and the peak maximum time (PMT) and are demonstrated in Table 3. The highest TM occurred in the control recipe containing 5% (w/w) added sucrose (49.8 ± 1.0 BU), whereas the lowest TM was detected in recipes containing 10% SD1 (35.2 ± 2.2 BU) and 10% SD2 (33.5 ± 2.7 BU). The addition of 5% SD1 or 5% SD2 showed the same torque maximum as the full-sugar control recipe did (44.0 ± 2.6 BU). PMT shows how fast the gluten network develops. The fasted formation was detected in the sugar reduced control (114.8 ± 2.4 s), while the longest development occurred, when 10% SD2 was incorporated. The addition of 5% sourdough caused the same PMT as the full-sugar control (160.0 ± 12.6 s).

3.2.1.5. Pasting properties. In order to investigate the impact of SD1 and SD2 on the starch gelatinization of sugar reduced burger bun dough, peak viscosity, breakdown, final viscosity and pasting temperature of these systems were determined by using RVA. The peak viscosity is the viscosity at which the starch swells to its maximum capacity. The results of the pasting properties are shown in Table 3. The sugar reduced control resulted in a significant higher peak viscosity than the full-sugar control (865.3 ± 78.8 cP). The addition of sourdough to the sugar reduced recipe showed the same peak viscosity as 10% added sucrose control. Breakdown is an indication of the stability of the system towards heat and sheering. The highest breakdown occurred in the 5% (w/w) added sugar control (324.0 ± 8.0 cP), whereas the full-sugar control showed the lowest stability (236.7 ± 7.4 cP). The addition of sourdough caused a lower stability than the sugar reduced control, but it withstood heat and sheering more than the full-sugar control, whereas the incorporation of 10% SD2 showed the same breakdown as the 10% (w/w) added sugar control system. The final viscosity represents the viscosity of the system after gelatinization and cooling. The addition of 10% SD1, 5% SD2 and 10% SD2 resulted in the same final viscosity as the full-sugar control (968.3 ± 73.9 cP), which was significantly lower than the final viscosity of the sugar reduced control (1225.0 ± 73.7 cP). No significant differences in pasting temperature were determined.

3.2.2. Impact of sourdoughs on burger bun quality

The effect of both sourdoughs on burger bun characteristics as well as their sensory attributes was investigated.

3.2.2.1. Specific volume. The highest specific volume was determined in the sugar reduced control containing 5% (w/w) added sucrose (4.47 ± 0.09 mL/g), whereas the lowest value occurred, when 10% SD2 (3.27 ± 0.06 mL/g) was incorporated (Table 4). The 5% (w/w) sucrose control differed significantly from the full-sugar burger bun (3.51 ± 0.07 mL/g), indicating less yeast inhibition with lower amounts of added sucrose. However, the full-sugar recipe did not show any significant differences to burger buns containing sourdough, regardless the type or amount of added sourdough. The incorporation of 10% SD2 showed the closest results to the 10% (w/w) added sugar control.

3.2.2.2. Crumb structure and ultrastructure. The most noteworthy parameter of the crumb structure was the area of cells. The biggest area of cells was determined, when SD1 (55.1 ± 0.8%–55.7 ± 1.9%) was incorporated into the burger bun system, whereas the full-sugar burger bun (53.2 ± 0.9%) and the addition of SD2 (52.5 ± 1.1%–53.4 ± 1.6%) resulted in the smallest area of cells (Table 4). The effect of the sourdough incorporation on the crumb ultrastructure compared with a full-sugar control and a no-added sugar sample is illustrated in Fig. 1 The incorporation of sourdough showed a significant change in crumb structure in form of a "film" which coated the starch granules. This coating was visible in full-sugar burger buns, but did not appear in buns without added sucrose.

3.2.2.3. Crumb texture. The crumb texture was measured using a texture profile analyser. The results are demonstrated in Table 4. No significant differences in cohesiveness, springiness or resilience amongst all samples were detected (data not shown). However, the addition of sourdough influenced the crumb hardness and chewiness of burger buns, as well as their staling rate. The addition of 10% SD2 caused the hardest crumb (5.24 ± 0.60 N) and showed no significant differences to the full-sugar control (4.39 ± 0.79 N). The softest crumb occurred in the reduced sugar burger bun control (1.73 ± 0.16 N). Furthermore, differences in chewiness between the controls and the buns containing sourdough were determined. Buns containing 10% SD2 showed the highest chewiness value (3.29 ± 0.44) and, again, did not differ significantly from the full-sugar control (2.68 ± 0.57). The less chewy crumb occurred in the sugar reduced control (1.24 ± 0.13). The staling rate indicates the retrogradation of the starch in the product. The highest staling rate was measured in the sugar reduced control burger bun (5.94 ± 1.00), which did not differ significantly from buns containing 5% SD1 (5.07 ± 0.82), 10% SD1 (5.18 ± 1.34) or 5% SD2 (5.39 ± 0.58). The full-sugar burger bun (4.58 ± 0.92) showed together with buns accommodating 10% SD2 (4.42 ± 0.67) the lowest staling rates.

3.2.2.4. Lightness of crumb and crust. The darkest crust colour appeared, when 5% SD1 (54.9 ± 2.2) or 5% SD2 (55.2 ± 2.1) was incorporated. The full-sugar control bun showed the palest crust amongst all samples (65.1 ± 3.3). The addition of 10% SD2 (59.4 ± 1.0) showed the same lightness value as the sugar reduced burger bun control (60.7 ± 0.9). Furthermore, sourdough influenced the lightness of the crumb. The more sourdough was incorporated into the system the darker was the crumb (Table 4).

3.2.2.5. Water activity and microbial shelf life. The water activity (a_w) is an important parameter which reflects the amount of free water available in the system. The lowest water activity was measured in the full-sugar control bun (0.914 ± 0.006), followed by the sugar reduced control (0.937 ± 0.005). The incorporation of sourdough, regardless the type and amount, increased the a_w-value significantly compared to the controls (Table 4). The shelf life profile is shown in Fig. 2. The longest shelf life was detected in burger buns containing 10% (w/w) added sucrose (10.0 ± 0.0 days), 10% SD1 (10.0 ± 0.0 days) or 10% SD2 (9.0 ± 0.0 days). The shortest shelf life was determined in the sugar reduced control (6.5 ± 0.7 days) and in buns with 5% SD1 (7.0 ± 0.0 days) or 5% SD2 (7.5 ± 0.7 days) (Table 4).

3.2.2.6. Sensorial properties. Sensory characteristics of a product are one of the most important parameters for consumer acceptance. The incorporation of sourdough revealed no significant differences in hardness amongst all samples. Furthermore, although all samples did not differ significantly from each other regarding sweetness intensity, absolute values indicated an amelioration of sweetness by sourdough addition. Regarding flavour and aroma, the full-sugar control showed significant higher intensities than the sugar reduced control. Burger buns containing SD2, regardless of the amount added, showed no significant differences in aroma compared to the controls. Furthermore, the incorporation of 10% SD1 or SD2 caused the same flavour intensity as the full-sugar control (Table 4).

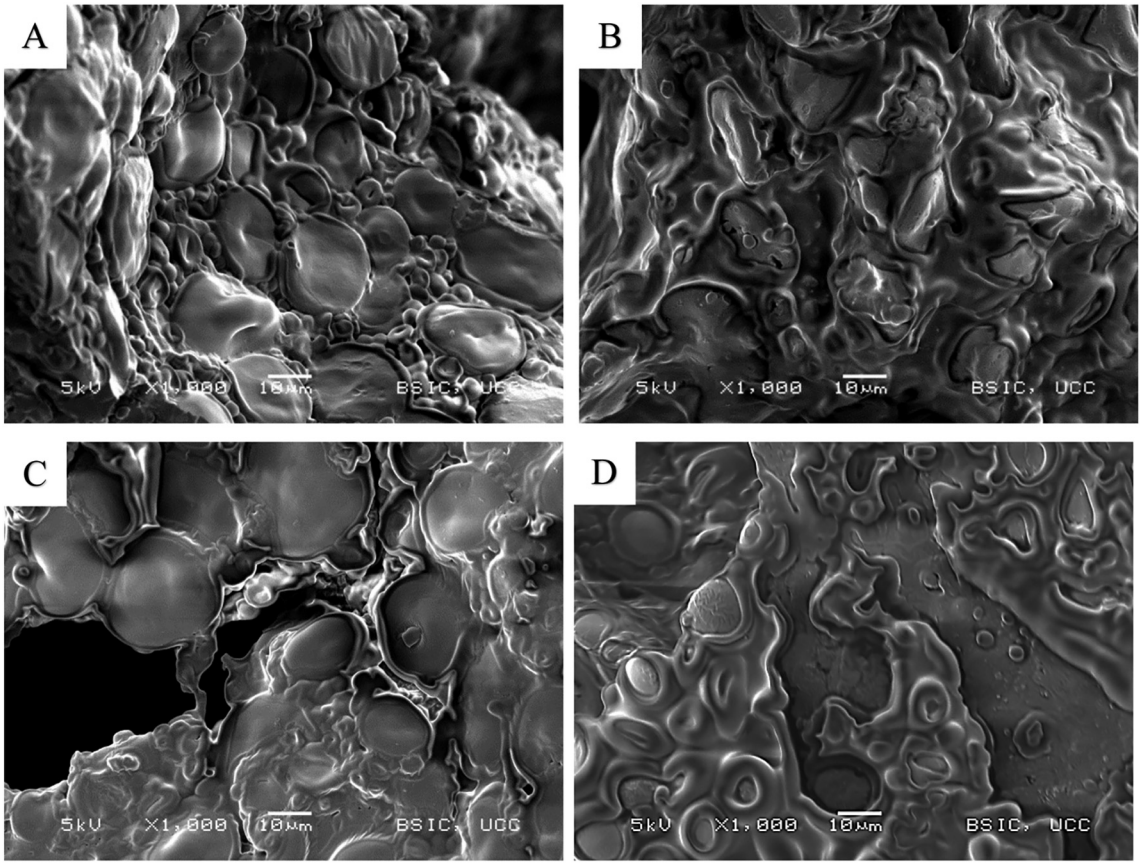

Fig. 1. Micrograph of burger bun crumb taken by a scanning electron microscope (SEM). (A) no added sucrose (×1000)*, (B) 10% added sucrose (full-sugar control) (×1000)*, (C) 10% sourdough 1, (D) 10% sourdough 2.
*Sahin et al. (2018)

4. Discussion

The application of sourdough in baked goods illustrates a long-standing tradition to enhance flavour and textural properties of the end-product. Previously, sourdough was never considered as a functional ingredient to reduce sugar in sweet baked goods. The results of this study showed high potential of sourdough fermentation by the mannitol producing strain *Leuconostoc citreum* TR116 for the production of sugar reduced burger buns. Before the incorporation of sourdough to sugar reduced burger buns, the suitable fermentation time was investigated, evaluating the microbial growth, acidification and quantifying sugars, mannitol, lactate and acetate.

The microbial growth in SD1 and SD2 over time did not differ significantly from each other, except for time point 48 h, where the cells of SD1 entered the death phase. The starting point of the stationary phase of microbial growth after 12 h of fermentation occurred most likely due to growth inhibition by the acidy environment (pH 4.5) (Hemme and Foucaud-Scheunemann, 2004). The added fructose in SD2 served as an additional fermentable substrate which prolonged the microbial stationary phase of growth compared to SD1 (Wisselink et al., 2002).

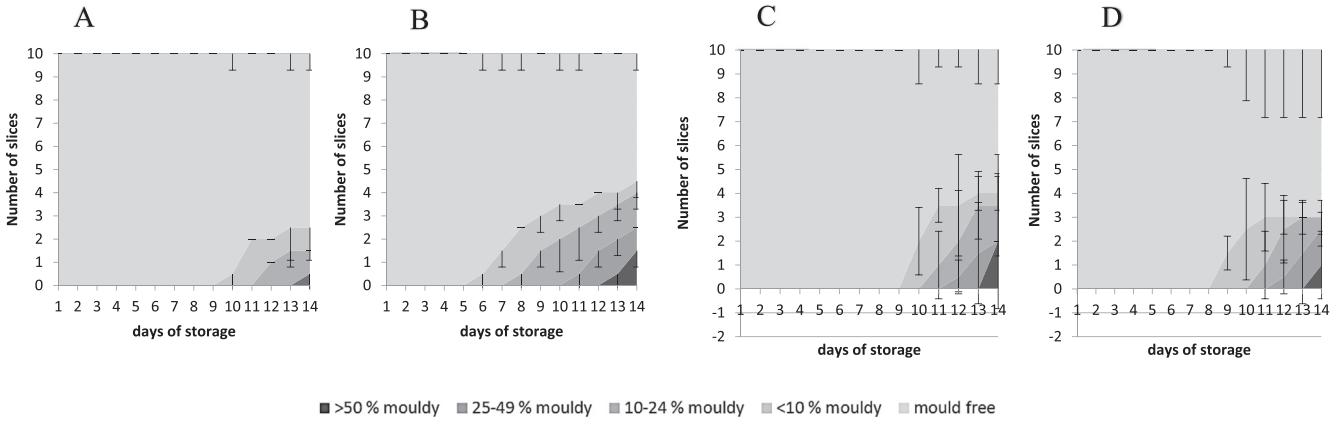

Fig. 2. Shelf life profile of burger buns containing (A) 10% added sucrose (full sugar control), (B) 5% added sucrose (sugar-reduced control), (C) 10% sourdough 1 and (D) 10% sourdough 2. The number of slices for each mould group ("mould-free", " < 10% mouldy", " < 10–24% mouldy", " < 25–49% mouldy" and > 50% mouldy") was counted on each day over 14 days. The graph shows mean values with standard deviations as error bars.

Furthermore, heterofermentative LAB, such as *Leuconostoc citreum* TR116, preferably produce acetate in the presence of fructose which results in the regeneration of one extra ATP (Saha and Racine, 2011). This provides additional energy for the cell and, hence, could contribute to the extended survival (Gänzle, 2015).

During fermentation, the pH dropped from 5.5 (initial pH) to 4.2 (pH after 48 h fermentation) in both sourdoughs, due to the production of organic acids, such as lactate and acetate. Although both sourdoughs had the same ν_{max} and pH profile, the TTA values of SD1 and SD2 showed significant differences. These differences are most likely related to the buffering capacity of the produced acids, mainly acetic acid. After 48 h of the fermentation the starting point of titration was in both sourdoughs a pH of 4.2. The titration was conducted towards pH 8.5, while mainly the buffer range of acetic acid (3.75–5.75) compared to lactic acid (2.86–4.86) was traversed (Chang, 2005). Considering that the concentration of acetic acid in SD2 is four fold of the amount determined in SD1, a higher TTA occurred in SD2. Due to the presence of little amounts of fructans, which got broken down into fructose during fermentation (Escrivá and Martínez-Anaya, 2000), small concentrations of mannitol and acetate arose in sourdough with no added fructose.

It is noteworthy that the increased concentration of acetate during fermentation correlated positively with the amounts of mannitol detected in both sourdoughs (SD1: $r = 0.89$, $P < 0.005$; SD2: $r = 0.98$, $P < 0.001$). Hereby, fructose acts as an electron acceptor and gets enzymatically reduced to mannitol by a mannitol-dehydrogenase, while regenerating the co-factor NAD^+. Acetate is formed from acetyl-phosphate by an acetate kinase (Gänzle, 2015). Evidently, acetate production is connected to fructose reduction to mannitol. The reduction of fructose to mannitol in SD2 showed a yield of 86.8%. It has been reported that the yield of mannitol production by *Leuconostoc species* depend on the species and cultivation conditions, such as medium composition, temperature and pH, and can vary between 26% and 90% (Carvalheiro et al., 2011; Erten, 1998; Otgonbayar et al., 2011; Saha and Racine, 2011; Von Weymarn et al., 2002). Taking into account that sourdough is a complex system a yield of 86.8% can be considered as high. Furthermore, the production of other metabolites, such as lactate and acetate, also depend on environmental conditions the microorganism is exposed to and on the strain itself. Hence, after 48 h fermentation a lower concentration of metabolites in SD2 (1:0.34:0.15 (mannitol:lactate:acetate) in mole) than theoretical possible (1:0.5:0.5 (mannitol:lactate:acetate) in mole), occurred (Saha and Racine, 2011). Lower amounts of acetate were determined either due to a potential lack of ADP, which is needed to convert acetyl-phosphate into acetate, or, more likely, due to stress in form of a low extracellular pH, since pH is the most important factor, which influences microbial growth and metabolite production (Pimentel et al., 1994). At the same time mannitol production is still implemented, most likely due to the fact that mannitol, like other polyols, protect the living cell by, for example, ensuring no structure changes of membrane lipids and other proteins at low water activity, which in turn can also be responsible for the longer stationary phase of microbial growth (Wisselink et al., 2002).

The sugar profile indicates an increase in maltose and glucose during the first 24 h in both sourdoughs, followed by a fluctuation or no change in the concentrations. The increase can be explained by the starch degradation by amylases in the flour during fermentation, resulting in maltose and glucose molecules, also reflected by the increase in glucose concentration during the stationary phase of microbial growth (after 18 h). The followed fluctuation occurred due to a decrease in maltose content. This reduction of maltose concentration is putatively due to, firstly, a further breakdown of maltose into glucose by β-amylases in the flour and, secondly, the metabolisation of maltose by the strain.

The application of both sourdoughs in a burger bun system showed different influence on dough and burger bun quality. The evaluation of the burger bun dough fermentation showed a decrease in maximum height of gaseous release (Hm') due to sourdough application. The incorporation of sourdough is known to change the dough structure due to hydrolysis of starch and modification in gluten network by naturally acidification (Arendt et al., 2007). A decreased Hm', when sourdough was incorporated into the system, occurred most likely because the dough network became weaker with sourdough addition. The decrease of elastic parts of the dough, represented by an increase of the damping factor, also proved the weakening effect of sourdough on the burger bun dough. Hence, the entrapment of the gas cells in the dough system was less efficient and resulted in lower dough rise during proofing. Furthermore, the prolonged PMT and lower TM of the gluten network, when sourdough was incorporated, reinforce this theory of a weak dough. The acidification of the dough causes a positive net charge and enhances the protein solubility, which contributes to an unfolding of the gluten. This change in tertiary structure leads to the exposure of hydrophobic groups which do not form new bonds due to strong intermolecular interactions (Galal et al., 1978; Takeda et al., 2001; Wehrle et al., 1997). The entangled protein network, in turn, increases the resistance to elongated extension (Masi et al., 2001). Furthermore, the micrographs (Fig. 1) of the burger bun crumbs showed clearly a film coating the starch granules, when 10% sugar (control) or 10% sourdough was incorporated into a sugar reduced system. This “film” represents, most likely, the fully hydrated and weakened gluten network in a more viscous dough system (Amend and Belitz, 1991; Bache and Donald, 1998).

Seemingly, the addition of sourdough did not just weaken the gluten network, it also inhibited starch gelatinization due to starch degradation reflected by a decrease in peak and final viscosity, as well as the breakdown, during pasting (Bertolini et al., 2000). Furthermore, lactate has been discovered to increases the solubility of amylopectin and hence decreases the viscosity of the dough system (Shandera and Jackson, 1996).

The weakening of the gluten network and the enhanced degradation of starch resulted in a lower specific volume of the burger buns, and thus a denser and harder crumb structure, which is connected to the increased chewiness. Usually sourdough is known to increase specific volume in a bread system (Corsetti et al., 2008). Due to the presence of 5% (w/w) added sucrose in the system, the dough structure is additionally weakened, since sugar itself inhibits the gluten network development (Sahin et al., 2017). Hence, the weak protein network of the dough, as well as the inhibited structure formation of starch during baking could cause the loss of produced CO_2. The addition of sourdough, especially 10% SD2, delayed staling of the burger bun. Proteases in the sourdough contribute in an increase of free H_2O in the system and thus enhance the activity of alpha amylases (Arendt et al., 2007) and increased the water activity. The microbial shelf life is often correlated to the water activity. A low water activity is known to result in a longer shelf life than products with a high a_w value. However, water activity did not correlate with microbial shelf life. The more sourdough was incorporated the longer was the shelf life. As a heterofermentative LAB strain *Leuconostoc citreum* produces organic acids and potentially ethanol (Choi et al., 2012; Corona et al., 2016), which can act as antimicrobial compounds. Hence, these antimicrobial compounds incorporated into the burger bun system in form of sourdough prolonged shelf life (Choi et al., 2012). Furthermore, the degradation of protein into amino acids contributed to browning reaction and resulted in a darker crust and crumb (Martins et al., 2000). Free amino acids also contribute to taste, flavour and aroma of the final product. Interestingly, sensory evaluation of sourdough burger buns compared to the full-sugar control showed no significant differences in hardness (bite), sweetness and sourness. It has been reported that some amino acids contribute to sweetness, sourness, bitterness or saltiness (Kato et al., 1989). Furthermore, amongst all sourdough burger buns, the incorporation of SD2 resulted in the highest sweetness. SD2 contained a higher amount of mannitol, which has a sweetness of 50–70% relative to sucrose. Sahin et al. (2018) demonstrated that sugar replacement by commercial mannitol showed, regardless the amount, the same

sensorial properties as a 10% (w/w) added sugar control.

A reduction of specific volume, a denser crumb structure, a prolonged shelf life, a brown crust colour and sweetness are typical burger bun attributes, and difficult to maintain during sugar reduction. The incorporation of sourdough fermented by *Leuconostoc citreum* TR116, as a high mannitol producer, showed high potential to ameliorate the losses of the named quality characteristics. Especially sourdough containing higher amounts of mannitol and lower amounts of lactate benefited the dough properties and burger bun quality. Hence, the incorporation of 10% SD2 into a sugar reduced burger bun is promising and highly recommended. An increase in mannitol production could be achieved by setting up a fed-batch sourdough fermentation, incorporating more fructose into the system over time.

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Conflict of interest

The authors declare that they have no conflict of interest.

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