Enzymatic and microbial conversions to achieve sugar reduction in bread

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Abstract

A standard level of sugar addition to bread is 2 % (flour base) but sweet baked goods including hamburger buns, hot dog buns and some sandwich bread contain more than 10 % sucrose. This study aimed to provide an integrated assessment of different strategies for sugar-reduced bread by using isomaltooligosaccharides (IMO) as bulk sweetening agent, polysaccharide hydrolases to generate sugars from flour polysaccharides, and sourdough. Trained panel sensory analyses of the intensity of sour and sweet tastes were compared to the concentration of organic acids and the sugar concentration of bread. Sourdough fermentation reduced the sweet taste intensity of bread produced with 9 % sucrose. This effect was more pronounced with *Leuconostoc mesenteroides*, which converts fructose to mannitol with concomitant production of acetate. Addition of up to 20 % sourdough fermented with *Weissella cibaria* 10M, which does not produce mannitol and less acetate when compared to *L. mesenteroides*, did not substantially reduce the sweet taste intensity. Bread produced with 9 % IMO tasted less sweet than bread prepared with 9 % sucrose but partial replacement of sucrose with IMO maintained the sweet taste intensity. Addition of 4.5 % IMO in combination with *W. cibaria* sourdough, amyloglucosidase and the fructosidase FruA enabled production of bread with 50 % reduced sucrose addition while maintaining the sweet taste intensity. In conclusion, the single use of a sweet bulking agent, of amyloglucosidase or fructanases or the use of sourdough alone, did not maintain the sweet taste intensity of sugar-reduced bread, however, a combination of the three approaches allowed a reduction of sucrose addition without reducing the sweet taste intensity.

Keywords: sucrose, sugar replacement, sweet taste, isomalto-oligosaccharides, sourdough, dextransucrase.

1 Introduction

A standard level of sugar addition to bread is 2 % (flour base). At this level, the sucrose addition enhances the CO_2 production by yeasts and flavour formation in the Maillard reaction but has very little influence on the taste of the final product (Sahin, Zannini, Coffey, & Arendt, 2019). Sweet baked goods including cakes, biscuits, sweet breakfast rolls and muffins, but also hamburger or hot dog buns and some sandwich bread, contain more than 10 % sucrose. At this level of addition, sucrose imparts a sweet taste to the product and also increases the calorie density and the glycaemic index of the products (Sahin, Zannini, et al., 2019). Sugar reduction has become a priority for the baking industry in order to meet consumers' demands (Anonymous, 2020; Green, 2017) and to comply with public health policy or taxation law (Clarke, O'Donnell, MacMenamin, Charleton, & O'Malley, 2020; Public Health England, 2019).

Sugar contributes to the sweet taste of baked goods and also fulfils technological functionalities during the baking process (Clemens et al., 2016). Strategies to reduce sugar content in sweet baked goods include the use of alternative sweet-tasting bulking agents, or a combination of high intensity sweeteners and bulking agents (Ghosh & Sudha, 2012; Sahin, Zannini, et al., 2019). Sweet tasting bulking agents for use in baking include sweet-tasting but indigestible polyols (Ghosh & Sudha, 2012) or isomalto-oligosaccharides (IMO) (Ruiz-Aceituno et al., 2018). IMO consist of mainly α-(1→4) and α-(1→6) linked glucose moieties. Depending on the method of production, the degree of polymerization ranges from 2 to more than 10 (Madsen, Stanley, Swann, & Oswald, 2017). Commercial IMO preparations taste sweet (Ruiz-Aceituno et al., 2018) and are also partially indigestible and thus reduce the caloric density of the product (Hu, Heyer, Wang, Zijlstra, & Gänzle, 2020; Hu, Winter, Chen, & Gänzle, 2017). Alternative approaches to reduce the sugar content of bread include the use of enzymes to convert flour

polysaccharides to sugars (Patent No. EP3266318A1, 2017; Patent No. WO 2017/220864 A1, 2017; Loponen & Gänzle, 2018), however, the impact of fructanases or amylases on the sweet taste of baked goods has not been evaluated experimentally.

Sourdough or sourdough products are increasingly used in baking applications to improve product quality, and to replace antifungal, texture-forming or taste-active additives (Arendt, Ryan, & Dal Bello, 2007; Belz, Ryan, & Arendt, 2012; Gänzle, 2014; Gobbetti et al., 2019; Zhao, Kinner, Wismer, & Gänzle, 2015). Sourdough fermentation also accumulates sweet tastants. Most heterofermentative lactic acid bacteria convert fructose to mannitol (Gänzle, 2014) and the accumulation of mannitol during sourdough fermentation was suggested as an alternative strategy for sugar reduction in bread (Sahin, Rice, et al., 2019). The formation of mannitol in heterolactic metabolism, however, is inevitably linked to the formation of acetic acid in a molar ratio of 2 mannitol : 1 acetate (Gänzle, 2014). Acetic acid not only imparts sour taste but also is an odorant with a flavour threshold of less than 1 mmol / kg (Hansen & Schieberle, 2005) that negatively impacts bread quality when present in excess concentrations. In contrast to most heterofermentative lactic acid bacteria, *Weissella cibaria* and *Weissella confusa* do not produce mannitol (and acetic acid) from fructose (Galle, Schwab, Arendt, & Gänzle, 2010), making *Weissella* species suitable candidates for high-sucrose sourdough fermentation without negative impact on product quality (Galle et al., 2012).

Sourdough fermentation with glucansucrase-positive LAB accumulates IMO in sourdough and sourdough bread (Schwab, Mastrangelo, Corsetti, & Gänzle, 2008). Species of the genera *Leuconostoc*, *Weissella*, *Liquorilactobacillus* and *Limosilactobacillus* generally exhibit glucansucrase activities (van Hijum, Kralj, Ozimek, Dijkhuizen, & van Geel-Schutten, 2006; Zheng et al., 2020) and, with exception of liquorilactobacilli, are frequently identified in sourdough microbiota (Gänzle & Zheng, 2019; Van Kerrebroeck, Maes, & De Vuyst, 2017).

While some of the process conditions that enhance IMO production in sourdough have been described, the final concentration in bread remains unknown and the impact on the sensory quality of bread has not been analysed (Hu & Gänzle, 2018).

Extracellular polysaccharide hydrolases are exceptional in lactic acid bacteria but *Amylolactobacillus* and *Lactobacillus* species express extracellular amylases (Gänzle & Follador, 2012) and a strain of *Lactobacillus crispatus* expresses the cell-wall bound extracellular fructanases FruA that hydrolyses fructans in wheat and rye during sourdough fermentation (Li, Loponen, & Gänzle, 2020).

This study aimed to provide an integrated assessment of different strategies for sugar-reduced bread by using IMO as bulk sweetening agent, polysaccharide hydrolases to convert flour polysaccharides to sugars, and sourdough fermented with the dextransucrase-expressing *Leuconostoc mesenteroides* FUA3090 and *Weissella cibaria* 10M. Bread was characterized with regards to the sweet and sour taste intensity, the presence of oligosaccharides, and the concentration of sugars and organic acids as sweet and sour tastants, respectively.

2 Materials and Methods

2.1 Strains and culture conditions

Leuconostoc mesenteroides FUA3090, an dextransucrase-producing isolate from kvas (Dlusskaya, Jänsch, Schwab, & Gänzle, 2008) and *Weissella cibaria* 10M, a dextransucrase-producing isolate from sourdough (Schwab et al., 2008) were routinely propagated in modified de Man, Rogosa, Sharpe agar containing 10 g / L maltose, 5 g / L glucose, 5 g / L fructose, 10 g / L peptone, 5 g / L yeast extract, 5 g / L beef extract, 4 g / L K_2HPO_4, 2.6 g / L KH_2PO_4, 0.5 g / L L-cysteine hydrochloride, 1 g / L Tween 80, 0.05 g / L $MnSO_4$ monohydrate, 0.1 g / L

anhydrous $MgSO_4$, 10 g / L and malt extract, 15 g L/ agar. Strains were incubated at 30 °C for 24 h unless otherwise noted.

2.2 Lab-scale sourdough fermentation

L. mesenteroides FUA3090 and *W. cibaria* 10M were grown for 24 h at 30 °C in 10 mL mMRS broth, washed twice with sterile tap water and resuspended in 10 mL sterile tap water. Tap water was used as a food-grade bread ingredient; the osmolarity of tap water in Edmonton, equivalent to 170 – 200 mg $CaCO_3$ / L (www.epcor.com), is sufficient to maintain bacterial viability and activity during culture preparation. Depending on the bread recipe, part of the sucrose and isomaltooligosaccharides (IMO) were added to the sourdough as detailed in Table 1. Lab-scale sourdough fermentations were started by mixing this inoculum with 10 g of flour (Robin Hood Bread flour homestyle white, Smucker Foods, Markham, ON, Canada), followed by incubation at 20 °C for 24 h.

2.3 Bread preparation

Sourdough breads were produced by mixing 80 g wheat flour (Robin Hood bread flour homestyle white), 35 g water, 2 g dried yeast (Fleischmann's), 0.6 g vital wheat gluten (Bobs Red Mill), 9 g sugar, 5 g canola oil, and 1.6 g salt and sourdough consisting of 20 g flour and 20 g water for a final dough yield of 155. All ingredients were bought in a local supermarket. Unless otherwise stated, sourdough bread was prepared with 20 g fermented flour per 100 g flour in the bread recipe. Straight dough bread without sourdough was produced in the same way but with addition of 55 g of water per 100 g flour to the bread dough and without sourdough addition. For the bread production, the ingredients were mixed in a spiral kneader (1 minute at level 1 and 7 minutes at level 4), followed by resting for 1.5 hours at 20 °C. Then, the dough was divided into 140 - 160 g loafs and proofed in aluminium pans for 1.5 h and 20 °C. Breads were baked at 177 °C for 11 minutes and cooled at room temperature for 2 h prior freezing. For

the sensory analysis, six batches of bread were produced, each including four different recipes (Tab. 1). Set 1 was composed of the reference bread (straight dough control bread; 9 % sugar), bread without sugar (straight dough, 0 % sugar), and two sourdough breads produced with *L. mesenteroides* FUA 3090 and *W. cibaria* 10M, respectively, as starter culture. Breads from sets 2 and 3 were produced with a constant addition of sugars but different amounts of sucrose and isomaltooligosaccharides (Vitafibre, BioNeutra, Edmonton). The total sugar concentration was 9 % consisting of sucrose, 6 % sucrose and 3 % IMO, 3 % sucrose and 6 % IMO, or 0 % sucrose and 9 % IMO. Breads with sucrose and IMO were produced with or without addition of 20 % sourdough fermented with *W. cibaria* 10M. Set 4 contained sourdough breads started with *W. cibaria* 10M and different amounts of sourdough addition (0 %, 10 %, 20 %, and 40 %). Set 5 was composed of breads with 20 % sourdough started with *W. cibaria* 10M and different amounts of sucrose (0 %, 3 %, 6 %, and 9 %). Set 6 contained the reference bread (straight dough control bread (9 % sugar) and three sugar reduced (-50 %) sourdough breads (20 % sourdough) with isomaltooligosaccharide addition (see Table 1), with and without addition of 0.05 g /100 g flour amyloglucosidase (Novozymes, Franklington, NC, U.S.A) and 1 g/ 100 g flour fructanase FruA (Oy Karl Fazer Ab, Vantaa, Finland) used alone or in combination.

2.4 Acidification and development of lactic acid bacteria in sourdoughs

Viable cell counts of lactic acid bacteria (LAB) were determined before and after fermentation by diluting sourdough 1:10 with 0.1 % peptone followed by surface plating on mMRS agar, followed by incubation for 3 d at 30 °C. Acidification was measured by analysing the pH values of sourdoughs before and after 24 h of fermentation by mixing 1 g sourdough with 9 ml of sterile 18 MΩ water.

2.5 Sensory analysis of bread with a trained panel

The protocol for sensory analysis of bread was approved by the Human Research Ethics Board of the University of Alberta (Study ID Pro00036093). A profile test of the bread crumb was performed by a trained panel. Bread samples were presented in cups coded with 3-digit random numbers. Prior to sensory analysis, screened panelists (9 female and 3 male, age: 22 – 46) completed six training sessions for the following attributes assessed on 10 cm scales: sweet (sugar as reference), salty (salt as reference), sour (lactic acid as reference), umami (monosodium glutamate as reference), bitter (caffeine as reference), and chewy (toast bread for absent and rye bread for extreme). In total, panelists evaluated six sets (Table 1), each including four different breads. For each standard (e.g. sweetness) panelists were provided with three reference breads (e. g. 0 % sugar, 7.5 % sugar, 15 % sugar). Sensory analysis was conducted in individual sensory booths at room temperature. The performance of panellists was verified by the ANOVA model ~ Sample + Panelist + Rep + Sample:Panelist + Sample:Rep + Panelist:Rep.

2.6 Quantification of organic acids and monosaccharides in sourdoughs and bread samples

Lactic acid, acetic acid, glucose, and fructose concentrations in sourdough and bread samples were quantified by HPLC. Sourdough or bread samples were diluted and mixed 1:5 with 18 MΩ water. Samples were heated for 3 h at 80 °C followed by centrifugation (3000 x g, 5 min.). Then, supernatants were diluted 1:1 with 7 % perchloric acid. Samples were stored at 4 °C overnight and filtered with a 0.45 µm filter before HPLC analysis. HPLC analyses were done on an Agilent 1200 system using an Aminex HP87X column coupled to an RI detector and eluted with 5 mmol / L H_2SO_4 in 18 MΩ water at 0.4 ml / min.

2.7 Analysis of oligosaccharides in sourdoughs and sourdough bread

Oligosaccharides in bread and sourdough samples were quantified by high performance anion exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD, Dionex,

Oakville). Sample (0.25 g) was mixed with 1 ml phosphate buffer followed by heating for 2 h at 80 °C. After centrifugation to remove solids, samples were diluted 10 times with 18 MΩ water. Oligosaccharides in diluted samples were separated on a Carbopac PA20 column coupled to an ED40 chemical detector (Dionex, Oakville, Canada) that was eluted with water (A), 0.2 mol / L NaOH (B) and 1 mol / L NaAcetate (NaOAc) (C) at 0.2 ml / min with the following gradient: 0 min, 68.3 % A,30.4 % B and 1.3 % C; 30 min, 54.6 % A, 30.4 % B and 15.0 % C; 50 min, 46.6 % A, 30.4 % B and 23 % C; 95 min, 33.3 % A, 30.4 % B and 36.3 % C; 95.1 min, 63.7 % A and 36.3 % C; 100 min, 50 % A and 50 % C; 105min, 10 % A, 73 % B and 17 % C; 105.1 min, 33.3 % A, 30.4 % B and 36.3 % C; 111 min, 10 % A, 73 % B and 17 % C; followed by re-equilibration.

2.8 Statistical analysis

Breads used for sensory analysis were prepared once and replicated twice by the panelists. Values for chemical data were performed in triplicate analyses from one batch of bread.

All data were analysed by one way variance analysis (ANOVA) and Tukey's HSD test was used for post hoc analysis. Principal component analysis (PCA) was performed on sensory data to describe bread samples and associations between sensory attributes. Multiple factor analysis (MFA) was performed on sensory and chemical data to describe associations between sensory attributes and the concentration of tastants. In the loading plot showing the sample differences, each sample is circled by a 95 % confidence ellipse generated by virtual panels using Bootstrap techniques. ANOVA models were performed using XLStat version 2020.4.1 at a significance value of $p \leq 0.05$. The PCA and MFA were performed using R version 3.5.2 (R Core Team, 2018).

3 Results

206 ### 3.1 Microbial growth and acidification during sourdough fermentation

207 To determine microbial growth and metabolism in sourdough, the viable cell counts and the pH
208 value were determined before and after fermentation (Table S1 of the online supplementary
209 material). During 24 h of fermentation, *L. mesenteroides* and *W. cibaria* grew from initial cell
210 counts ranging from 8.0 x 10^7 to 8.7 x 10^8 CFU/ g sourdough to cell counts ranging from 1.6 x
211 10^8 to 1.9 x 10^9 CFU/ g and pH decreased from 5.3 – 6.0 to less than 4.0. The observation of a
212 uniform colony morphology that matched the colony morphology of the respective strains used
213 as inoculum confirmed that both strains dominated the sourdough microbiota in all experiments.

214 ### 3.2 Impact of sucrose and sourdough fermented with *L. mesenteroides* FUA3090 and
215 *W. cibaria* 10M on the taste attributes of bread

216 *L. mesenteroides* FUA3090 and *W. cibaria* 10M were used as starter cultures in 80 g lab-scale
217 sourdough fermentations. The impact of the cultures on the bread flavour was analysed by
218 sensory analysis and by quantification of monosaccharides and organic acids, and by
219 determination of the oligosaccharide profile of breads.

220 A trained sensory panel assessed the two sourdough breads with 9 % sucrose (one with *L.*
221 *mesenteroides* FUA3090, one with *W. cibaria* 10M as starter culture), in comparison to bread
222 without sucrose (control) and a reference bread with sucrose but without sourdough. Linear
223 discriminant analysis of the sensory attributes of breads clearly separated all four breads (Figure
224 1A). The loading plot indicated that bread with sucrose was characterized by sweetness and
225 chewiness. Sourdough bread produced with *L. mesenteroides* was characterized by sour taste
226 and reference bread without sucrose or sourdough was characterized by bitterness (Figure 1B).
227 Sourdough bread produced with *W. cibaria* as starter culture was significantly (P<0.05) sweeter
228 and less sour than sourdough bread produced with *L. mesenteroides.* The intensity of the sweet
229 and sour taste of the bread, and the chewiness is depicted in Figure 2. The intensity of sweet

taste was highest for bread with sucrose and the intensity of sour taste was highest for sourdough bread produced with *L. mesenteroides* (Figure 2).

To correlate the sensory characteristics of bread with the concentration of sweet and sour tastants, fructose, glucose, lactic acid, and acetic acid concentrations in bread were quantified (Table 2). Sucrose was not detected in any of the breads, suggesting hydrolysis by yeast invertase at the dough stage. Bread with 9 % sucrose added at the dough level contained approximately 150 mmol of (glucose + fructose) / kg, corresponding to a sugar concentration of about 3 % or less than 1/3rd of the amount of sucrose added at the dough stage. The monosaccharide concentrations in sourdough bread were not different (P>0.05) from reference breads but the concentrations of lactic and acetic acids were higher (P<0.05) (Table 2). Bread with sourdough fermented with *L. mesenteroides* FUA3090 revealed the highest lactic acid content compared to other breads whereas the acetic acid content was similar in both sourdough breads, independent of the starter applied.

L. mesenteroides FUA3090 and *W. cibaria* 10M both express dextransucrase during growth in sourdough, leading to the formation of panose-series oligosaccharides when maltose is present (Dlusskaya et al., 2008; Hu et al., 2017). The pattern of oligosaccharides in sourdough bread produced with *L mesenteroides* and *W. cibaria* is show in Figure 3, reference bread and bread with 3 % IMO is shown for comparison. The oligosaccharide pattern in bread produced with IMO shows the characteristic pattern of oligosaccharides in IMO, consisting of isomaltose-series and panose-series oligosaccharides (Hu, Winter, & Gänzle, 2020). Both strains produced panose-series oligosaccharides during growth in sourdough (Figure 3).

3.3 Impact of the amount of sugars and sourdough on the taste attributes of bread

To determine the amount of sourdough that significantly impacts the sweet and sour tastes of bread, bread was prepared with different amounts of sourdough fermented with *W. cibaria*

ranging from 0 to 40 % (Figure 4A). The addition of up to 20 % sourdough did not significantly change the intensity of sweet or sour taste while addition of 40 % sourdough fermented with *W. cibaria* significantly decreased sweetness and significantly enhanced sourness of bread (Figure 4A).

The impact of sugar addition to bread was determined in three experiments; one analysing sourdough breads with increasing amounts of sucrose, a second analysing breads with 9 % sugar and a variable ratio of sucrose to IMO and a third analysing sourdough breads with 9 % sugar and a variable ratio of sucrose to IMO (Figure 4B). Sucrose addition enhanced the sweet taste when added at a level of 6 % or 9 % to bread dough. The use of IMO compensated for the reduced addition of sucrose in straight dough bread but not in sourdough bread. When partially substituting sucrose with IMO, bread produced with 6 % IMO and 3 % sucrose tasted less sweet than bread with 9 % sucrose (Figure 4B). Bread produced with 3 % IMO and 6 % sucrose tasted as sweet as the corresponding bread with 9 % sucrose. In sourdough bread produced with sucrose or IMO and sucrose, even partial replacement of sucrose with IMO reduced ($P<0.05$) the sweetness when compared to bread with addition of 9 % sucrose.

3.4 Sugar reduced sourdough bread

To evaluate the effect of enzyme addition on the sweet taste of bread, sourdough bread was prepared with *W. cibaria* 10M and addition of amyloglucosidase or fructanases. In these breads, sucrose was partially replaced with IMO to maintain a total sugar concentration of 9 % with a reduced sucrose content of 4.5 %. The sensory properties of sucrose reduced bread were compared to reference bread with 9 % sugar but without sourdough. Organic acid and sugar concentrations in the sourdoughs used for baking are shown in Table S2 of the online supplementary material. Linear discriminant analysis differentiated all four breads on the basis of their sensory properties (Figure 5).

The intensity of sweet and sour taste attributes of the four breads differed ($P<0.05$) (Figure 6). Reference bread was predominantly characterized by sweet taste and sucrose-reduced bread with sourdough but without enzymes was characterized mainly by sour taste (Figure 6). The addition of amyloglucosidase to sucrose-reduced sourdough bread did not significantly ($P>0.05$) alter the intensity of sweet and sour taste relative to sourdough bread without enzyme addition. Addition of fructanase, however, significantly ($P<0.05$) enhanced the sweet taste and enabled production of bread with an intensity of sweet and sour taste that was not different ($P>0.05$) from the reference bread (Figure 6).

The concentrations of organic acids, glucose and fructose in sucrose reduced breads is shown in Table 3. The concentration of glucose and fructose in reference bread matched the concentration in other batches produced with the same recipe (Table 2). The concentration of glucose and fructose was lower ($P<0.05$) in sourdough bread with reduced sucrose addition when compared to reference bread with 9 % sucrose while the concentration of organic acids was increased ($P<0.05$) (Table 3). The addition of amyloglucosidase increased ($P<0.05$) the concentration of glucose to levels that were equivalent to reference bread with 9 % sucrose; fructose concentrations were also increased ($P<0.05$) but not to the same level as the reference bread. The addition of fructanases increased ($P<0.05$) the concentration of fructose relative to the bread produced with sourdough but without enzymes (Table 3). Enzyme addition had no impact on the concentration of organic acids.

The oligosaccharide profiles of reference breads and sourdough breads with reduced sugar content is shown in Figure S1 of the online supplementary material. Bread produced with 3 % IMO again showed the characteristic oligosaccharide profile of the IMO preparation used. The addition of amyloglucosidase did not change this profile, suggesting that amyloglucosidase

preferentially hydrolyses α-(1→4) linkages of starch and maltodextrins rather than α-(1→6) linkages that are predominant in IMO (Figure S2 of the online supplementary material).

Principle Component Analysis was used to depict correlations between the sensory properties of bread and the concentration of tastants (Figure 7). The concentration of lactic and acetic acids correlated to the intensity of sour, salty and bitter taste while the concentration of fructose and glucose correlated to the intensity of sweet taste and the chewiness of bread (Figure 7).

4 Discussion

This study assessed the use of bacterial and enzymatic conversions to enhance the sweet taste of bread, and to allow the reduction of sugar addition to sweet baked goods without compromising sweet taste. No single enzyme or additive was effective in substituting sugar without reducing the intensity of sweet taste of the product, but a combination of enzyme addition, replacement of sucrose with the sweet-tasting bulking agent IMO, and sourdough fermentation allows a reduction of sucrose concentrations by 50 % without reducing the sweet taste intensity.

The most prominent microbial metabolic activity with regards to sugar concentrations was the conversion of sugars to ethanol and organic acids. While the metabolism of lactic acid bacteria in sourdough is well described (Gänzle, 2014), the metabolism during dough mixing and proofing is rarely reported. If active sourdough is incorporated at 20 % or less of the formulation, the concentration of sugars and organic acids is determined by metabolic conversions in bread dough rather than the preceding sourdough fermentation (Li et al., 2020; Menezes et al., 2019; Quattrini et al., 2019). Yeast metabolism consumed up to 5 % sucrose at the dough stage and the remaining sucrose was hydrolysed to glucose and fructose by yeast invertase (Nilsson, Öste, & Jägerstad, 1987) if it was added at a level of 6 % of higher. The relative sweetness of glucose and fructose is 0.75 and 1.7, respectively (Moskowitz, 1971).

Accordingly, the relative sweetness of a 3 % solution of equal amounts of glucose and fructose is equivalent to 7 to 8 % sucrose. In bread with added sucrose, the fructose concentrations exceeded glucose concentrations 2 to 3 fold; this likely reflects partial hydrolysis of wheat fructans by yeast invertase (Loponen & Gänzle, 2018; Menezes et al., 2019).

Lactic metabolism in sourdough further reduced sugar concentrations, in keeping with the conversion of hexoses to lactic acid, CO_2 and ethanol or acetate, of fructose to mannitol, and of sucrose to dextran, isomaltooligosaccharides, or glucose (Gänzle, 2014). The contribution of these metabolites to the taste of bread was assessed by comparison of bread produced with *L. mesenteroides* FUA3090, which converts fructose to mannitol and accumulates panose-series IMO, to bread produced with *W. cibaria* 10M, which does not convert fructose to mannitol and produces a lower amount of panose-series oligosaccharides (this study, Galle et al., 2010). Despite similar sugar concentrations, the resulting bread produced with *L. mesenteroides* FUA3090 tasted sourer and less sweet than the bread produced with *W. cibaria* 10M, indicating that the production of sweet tasting metabolites by *L. mesenteroides* was more than compensated by the formation of increased amounts of acetate. This observation stands in apparent contrast to previous studies that suggested that mannitol formation by *Leuconostoc* species is a suitable tool for sugar replacement (Sahin, Rice, et al., 2019). Sahin and co-authors employed a trained panel to assess the overall quality of the “flavor” and the “aroma” of bread while the panelists in the present study were trained to assess the intensity of the individual taste qualities. When accounting for these differences, both studies provide the conclusion that the use of sourdough enhances taste intensity (this study, Sahin, Rice, et al., 2019). The present study demonstrates, however, that the increased taste intensity of sourdough bread produced with *Leuconostoc* species is attributable to salty and sour taste rather than sweet taste. It was surprising to note that a relatively minor difference in the concentrations of lactic and acetic

acids in bread produced with *L. mesenteroides* and *W. cibaria* had a significant impact on the sweet and sour taste of bread. Data on the sensory impact of lactic and acetic acids on the taste of sourdough bread is almost exclusively based on sourdough breads fermented with type I sourdoughs, which indicates that a molar ratio of lactic to acetic acids (fermentation quotient, FQ) of 3 to 4 corresponds to a high bread quality (Spicher & Stephan, 1993). The FQ of breads analyzed in the present study ranged from 1 to 2, suggesting that the sensory impact of lactic and acetic acids relates to their absolute concentration of lactic and acetic acids rather than the ratio of the two compounds.

IMO are produced from starch or sucrose and maltose by a combination of glucosyl hydrolases and transglucosidases to yield α-(1→4) and α-(1→6) linked gluco-oligosaccharides with a degree of polymerization of 2 to 10 (Casa-Villegas, Marín-Navarro, & Polaina, 2018; van der Maarel, van der Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2002; van der Maarel & Leemhuis, 2013). Products that are currently commercially available differ with respect to the production methods, the content of glucose and maltose, and the degree of polymerization, but most include sweet tasting oligosaccharides and indigestible oligosaccharides that add to the dietary fibre content of foods (Goffin et al., 2011). The IMO product used in the present study, Vitafibre, has a relative sweetness of 0.3 to 0.5 and is about 50 % digestible (Hu, Heyer, et al., 2020; Hu, Winter, et al., 2020). Replacement of one third of the sucrose with IMO maintained the sweet taste at the level of the control with 9 % sucrose, but replacement of two thirds of the sucrose with IMO reduced the sweet taste intensity (this study). Considering the sugar content of IMO, this represents about half of what is needed to meet the requirements for labeling of energy / sugar reduced food products (CFIA, 2020; EFSA, 2020; Public Health England, 2019).

Amylolytic enzymes including amyloglucosidase are used as baking improvers to modify starch structure, and to generate reducing sugars to promote yeast activity, to enhance the Maillard

reaction, and to increase sweetness of bread (Tebben, Shen, & Li, 2018). Amyloglucosidase is an exo-active enzyme that releases glucose from the non-reducing end of starch; it has little impact on starch functionality but increases the content of reducing sugars (Tebben et al., 2018). Sourdough that includes *Lactobacillus* spp. that express the fructanases FruA is used commercially to hydrolyse wheat and rye fructans at the dough stage, which allows the production of low-FODMAP bread (Laatikainen et al., 2017; Li et al., 2020). Using both enzymes in combination enhanced the glucose and fructose concentrations of bread and enhanced the sweetness of bread relative to control bread produced with the same sourdough and the same sugar concentration. (Table 3 and Figures 6 and 7). Of note, although amyloglucosidase hydrolyses α-(1→6)-linked IMO (Pazur & Ando, 1960; Tanabe, Nakamura, & Oku, 2014), the oligosaccharide profile in IMO-supplemented wheat dough was not altered by addition of amyloglucosidase. The use of amyloglucosidase only was less effective when compared to the use of both enzymes, however, fructose is less likely to cause digestive discomfort in fructose malabsorbing individuals when it is associated with equal concentrations of glucose (Fedewa & Rao, 2014). Therefore, it may be preferable to use fructanases in association with amylases. The dose of the fructanase FruA as used in this study is sufficient to hydrolyse wheat fructans at the dough stage (Li et al., 2020), however, glucose accumulation in dough can be further increased by a combination of α- and β-amylases with amyloglucosidase, or by use of enzyme-active malt (Tebben et al., 2018).

The sensory analysis method used in the present study successfully quantified the intensity of basic tastes with trained panelists, however, consumer preference was not assessed and is subject for future studies. This is important in two respects: First, past studies demonstrated that a reduced intensity of a specific taste (sweet, salty) as assessed by a trained panel can be compensated by an enhanced intensity of other basic tastes without reducing consumer

preference (Sahin, Rice, et al., 2019; Zhao et al., 2015). Second, consumer preference of food products is not only influenced by the sensory properties of food but also by label information (Martínez Michel, Anders, & Wismer, 2011) and label claims pertaining to “low sugar”, “low calorie” or “high fibre” may enhance consumer preference despite or even because of a lower intensity of sweet taste.

In conclusion, the present study provides a comparative assessment of different strategies to reduce the sugar addition to bread without reducing the sweet taste intensity. The use of IMO as sweet bulking agent, the use of amyloglucosidase or fructanases or the use of sourdough alone did not enhance the sweet taste intensity of sugar-reduced bread to the same level as the control, however, a combination of the three approaches allowed a reduction of sucrose addition without reducing the intensity of the sweet taste. The results thus may provide guidance for the development of sugar-reduced baked goods.

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Figure legends

Figure 1: Principal component analysis (PCA) of sensory attribute intensities for bread without and with sugar addition, and without and with addition of 20 % sourdough fermented with *L. mesenteorides* FUA3090 or *W. cibaria* 10M. **Panel A**: Linear discriminant analysis of the sensory attributes of breads; ellipses depict the 95 % confidence interval. Non-overlapping confidence ellipses indicate significant differences among the breads ($P < 0.05$). **Panel B:** Loading plot showing the correlations among sensory attributes of breads; Breads are colour-coded as follows: **0S_1 (black):** Bread without sugar added (straight dough); **9S_1 (red):** Reference bread with 9 % sugar (straight dough control bread); **L (green):** Bread with 9 % sugar and sourdough (20 %) fermented with *L. mesenteroides* FUA3090; **W (blue):** Bread with 9 % sugar and sourdough (20 %) fermented with *W. cibaria* 10M.

Figure 2. Sweet and sour taste intensity and chewiness of bread produced with or without sucrose and with or without sourdough. Black bars, sweetness, white bars, sourness, gray bars, chewiness. Sourdough was fermented with *L. mesenteroides* FUA3090 or *W. cibaria* 10M; 20 % of the flour in the recipe, sucrose was added in a ratio of 1:2 (sourdough/dough). Bars representing the same sensory attribute differ significantly ($P<0.05$) if they don't share a common superscript.

Figure 3. Oligosaccharide profiles of reference bread, bread produced with 3 % isomalto-oligosaccharides and 6 % sucrose, and sourdough bread with 9 % sucrose and fermented with *L. mesenteroides* FUA3090 or *W. cibaria* 10M. Peaks were identified on the basis of external standards (maltose, isomaltose, panose), or on the basis of enzymatically synthesized isomalto-oligosaccharides and panose-series oligosaccharides (Hu, Heyer, et al., 2020; Hu et al., 2017). Chromatograms are offset by 0.1 µC. The x-axis was scaled to exclude monosaccharides, which

elute between 5 and 10 min but were quantified separately on an Aminex HP87X column coupled to an RI detector.

Figure 4. Sweet and sour taste intensity of bread produced with different amount of sourdough fermented with *W. cibaria* 10M **(Panel A)**, and sweet taste intensity of bread where sucrose was replaced with isomalto-oligosaccharides (IMO) (**Panel B). Panel A.** Sweetness (black bars) and sourness (white bars) of bread produced without sourdough, or with 10, 20, or 40 % addition of sourdough fermented with *W. cibaria* 10M. All breads were formulated with 9 % sucrose, where sourdough was used, sucrose was added in a ratio of 1:2 (sourdough/dough). **Panel B**. Sweet taste containing 0, 3, 6, or 9 % sucrose and 20 % sourdough fermented with *W. cibaria* 10M (black bars), different sucrose concentrations with balance to 9 % sugar addition provided by IMO but no sourdough (white bars), and different sucrose concentrations with balance to 9 % sugar addition provided by and addition of 20 % sourdough fermented with *W. cibaria* 10M (gray bars). Sucrose used in the bread recipe was added to sourdough and dough (Table 1), where applicable, IMO were added to sourdough and bread dough. Bars of the same colour representing the same sensory attribute differ significantly ($P<0.05$) if they do not share a common superscript.

Figure 5: Principal component analysis (PCA) of sensory attributes of bread without and with sourdough addition (*W. cibaria* 10M, 20 %, 24 h, 20 °C), and with and without addition of amyloglucosidase (0.05 g Amyloglucosidase (AMG) in 100 g flour) or fructanase FruA (0.05 g AMG/100 g flour and 1 g FruA/100 g flour). Shown is the linear discriminant analysis of the sensory attributes of breads; ellipses depict the 95 % confidence interval. Non-overlapping confidence ellipses indicate significant differences among the breads ($P < 0.05$). Breads are colour-coded as follows: **Ref (black):** Reference bread with addition of 9 % sugar (straight dough control bread); **W_6 (red):** Sugar-reduced bread (4.5 %) with addition of sourdough

fermented with *W. cibaria* 10M; **W_AMG (green):** Sugar-reduced bread (4.5 %) with addition of sourdough fermented with *W. cibaria* 10M and AMG; **W_AMG_F (blue):** Sugar-reduced bread (4.5 %) with addition of sourdough fermented with *W. cibaria*, 10M, AMG and FruA. 4.5 % isomalto-oligosaccharides / 100 g flour was used as bulking agent in sugar-reduced bread.

Figure 6: Sweet and sour taste intensity of bread produced with 9 % sucrose or with 4.5 % sucrose and addition of sourdough and enzymes as indicated on the x-axis. Black bars, sweetness, white bars, sourness. Sugar reduced breads were prepared with 20 % sourdough fermented with *W. cibaria* 10M, or sourdough and addition of amyloglucosidase AMG, or sourdough and addition of AMG and fructosidase FruA. Bars representing the same sensory attribute differ significantly ($P<0.05$) if they don't share a common superscript.

Figure 7: Multiple factor analysis (MFA) of sensory attributes and the chemical composition of sugar reduced breads produced with sourdough, or with sourdough and addition of enzymes. The loading plot shows the correlations among sensory attributes of bread and the concentration of tastants. **Ref:** Reference bread with 9 % sugar; **W_6:** Sugar-reduced bread (4.5 %) with 20 % *W. cibaria* 10M sourdough; **W_AMG:** Sugar-reduced bread (4.5 %) with 20 % *W. cibaria* 10M sourdough and addition of amyloglucosidase; **W_AMG_F:** Sugar-reduced bread (4.5 %) with 20 % *W. cibaria* 10M sourdough and addition of amyloglucosidase and fructanases.

642 **Table 1.** Overview of the experimental design**.**

Samples	Sourdough (%)[1)]	Sucrose (%)	
		in sourdough	in dough
Control (no sourdough, no sucrose)	-	-	-
9 % sucrose, no sourdough (reference)	-	-	9
W. cibaria 10M, 9 % sucrose	20	3	6
L. mesenteroides FUA3090 9 % sucrose	20	3	6
IMO[2)] 0 %, sucrose 0 %	20	-	-
IMO 3 %, sucrose 6 %	20	3 (1:2 IMO/sucrose)	6 (1:2 IMO/ sucrose)
IMO 6 %, sucrose 3 %	20	3 (2:1 IMO/sucrose)	6 (2:1 IMO/ sucrose)
IMO 0 %, sucrose 9 %	20	3	6
IMO 0 %, sucrose 0 %	-	-	-
IMO 3 %, sucrose 6 %	-	3 (1:2 IMO/sucrose)	6 (1:2 IMO/ sucrose)
IMO 6 %, sucrose 3 %	-	3 (2:1 IMO/sucrose)	6 (2:1 IMO/ sucrose)
IMO 0 %, sucrose 9 %	-	3	6
Control (no sourdough)	-	-	-
10 % sourdough addition (*W. cibaria* 10M)	10	3	6
20 % sourdough addition (*W. cibaria* 10M)	20	3	6
40 % sourdough addition (*W. cibaria* 10M)	40	3	6
0 % sucrose	20		
3 % sucrose	20	3	0
6 % sucrose	20	3	3
9 % sucrose	20	3	6
Reference bread with 9 % sucrose	-	-	9
W. cibaria 10M (no enzymes added), 4.5 % sucrose	20	3 (1:1 IMO/sucrose)	6 (1:1 IMO/sucrose)
W. cibaria 10M + 0.05 g AMG[3)], 4.5 % sucrose	20	3 (1:1 IMO/sucrose)	6 (1:1 IMO/sucrose)
W. cibaria 10M + 0.05 g AMG + 1g FruA[4)], 4.5 % sucrose	20	3 (1:1 IMO/sucrose)	6 (1:1 IMO/sucrose)

643 [1)] % of wheat flour used in bread recipe;
644 [2)] IMO , isomalto-oligosaccharides (Vitafiber, Bioneutra, Edmonton, Canada),
645 [3)] AMG, amyloglucosidase, Novozymes; Franklington, NC, U.S.A.
646 [4)] FruA, fructosidase from *Lactobacillus crispatus*, Oy Karl Fazer Ab, Vantaa, Finland
647

Table 2. Concentration of glucose, fructose, lactic acid, and acetic acid in bread produced with 20 % sourdough fermented with *W. cibaria* 10M or *L. mesenteroides* FUA3090. Shown are means ± standard deviations of triplicate analyses of bread. Values in the same column differ significantly ($p<0.05$) if they do not share a common superscript.

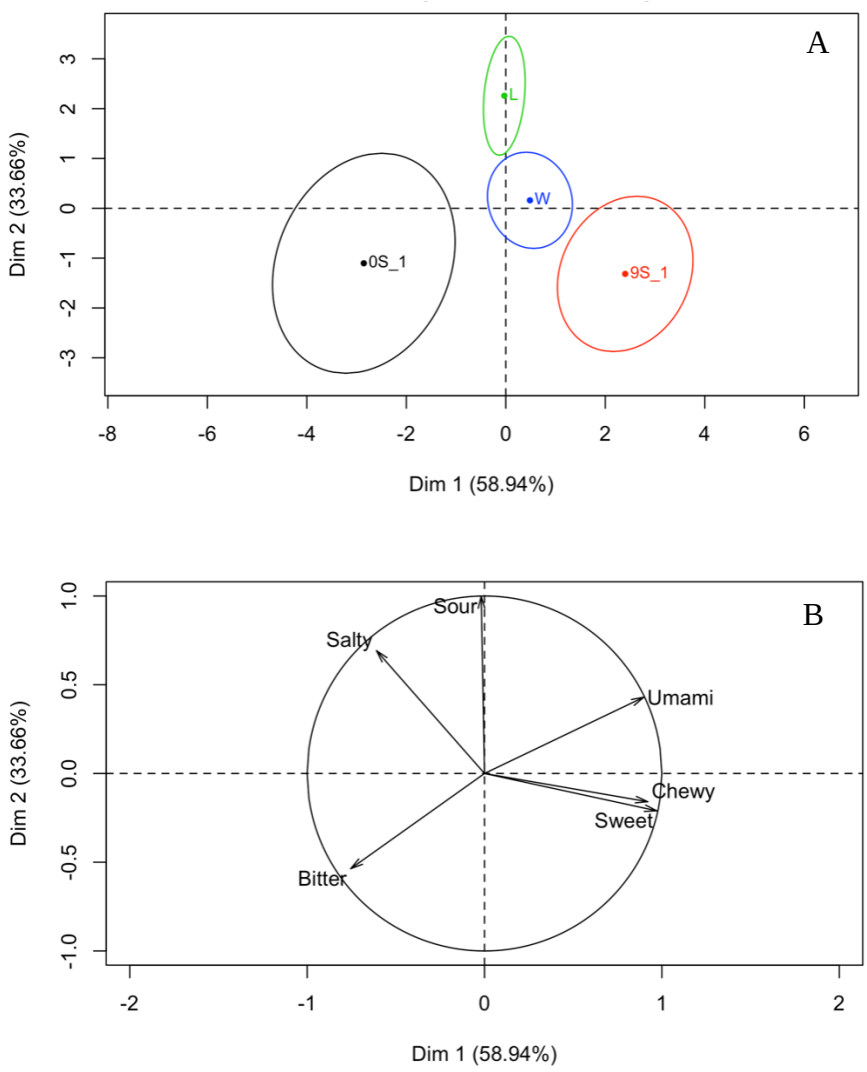
Bread samples	Carbohydrates [mmol/kg bread]		Acids [mmol/kg bread]	
	Glucose	**Fructose**	**Lactic acid**	**Acetic acid**
Reference bread (9 % sucrose)	44.8 ± 2.2^{a}	111.7 ± 20.7^{a}	13.6 ± 0.7^{c}	7.4 ± 2.9^{c}
L. mesenteroides FUA3090	40.5 ± 9.6^{a}	94.6 ± 18.8^{a}	22.7 ± 2.2^{a}	21.7 ± 1.5^{a}
W. cibaria 10M	32.8 ± 2.7^{b}	98.7 ± 19.6^{a}	17.4 ± 0.5^{b}	15.3 ± 1.9^{b}

Table 3: Concentration of glucose, fructose, lactic acid, and acetic acids in sucrose reduced bread (4.5 % sucrose) produced with addition of 4.5 % isomalto-oligosaccharides and 20 % sourdough fermented with *W. cibaria* 10M, and in reference bread produced with 9 % sucrose but without sourdough. Data are show as means ± standard deviation of triplicate experiments. Values in the same column that do not share a common superscript differ significantly ($P<0.05$).

Bread samples	Sucrose [g/100 g]	Carbohydrates [mmol/kg]		Acids [mmol/kg]	
		Glucose	Fructose	Lactic acid	Acetic acid
Reference	9	45.4 ± 4.1^{a}	122.6 ± 12.9^{a}	13.5 ± 2.5^{b}	7.2 ± 1.4^{b}
W. cibaria 10M	4.5	23.4 ± 3.9^{b}	67.4 ± 4.8^{c}	24.6 ± 0.4^{a}	20.5 ± 0.8^{a}
W. cibaria 10M + 0.05 g AMG[a)]	4.5	44.2 ± 3.8^{a}	84.5 ± 7.3^{bc}	24.4 ± 0.2^{a}	18.8 ± 1.1^{a}
W. cibaria 10M + 0.05 g AMG + 1 g Fructanase	4.5	45.6 ± 3.7^{a}	88.6 ± 3.1^{b}	22.7 ± 1.2^{a}	21.2 ± 1.2^{a}

[a)] AMG, amyloglucosidase

Figure 1:


A
L
W
0S_1
9S_1
Dim 2 (33.66%)
Dim 1 (58.94%)
-8
-6
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0
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2
1
0
-1
-2
-3

B
Sour
Salty
Umami
Chewy
Sweet
Bitter
Dim 2 (33.66%)
Dim 1 (58.94%)
1.0
0.5
0.0
-0.5
-1.0
-2
-1
0
1
2

Figure 2.

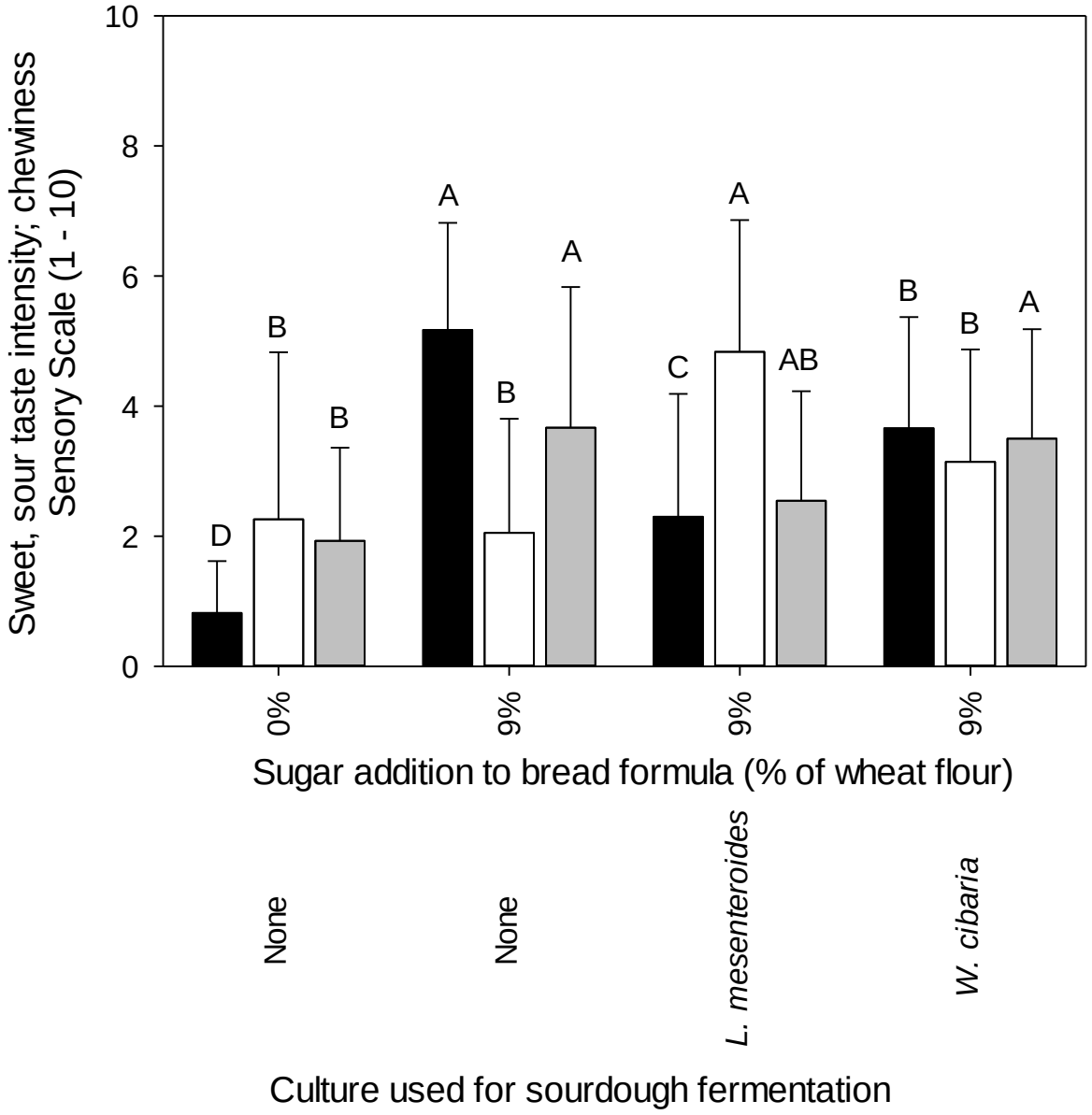

Sweet, sour taste intensity; chewiness
Sensory Scale (1 - 10)
10
8
6
4
2
0
D
B
B
A
B
A
C
A
AB
B
B
A
0%
9%
9%
9%
Sugar addition to bread formula (% of wheat flour)
None
None
L. mesenteroides
W. cibaria
Culture used for sourdough fermentation

Figure 3.

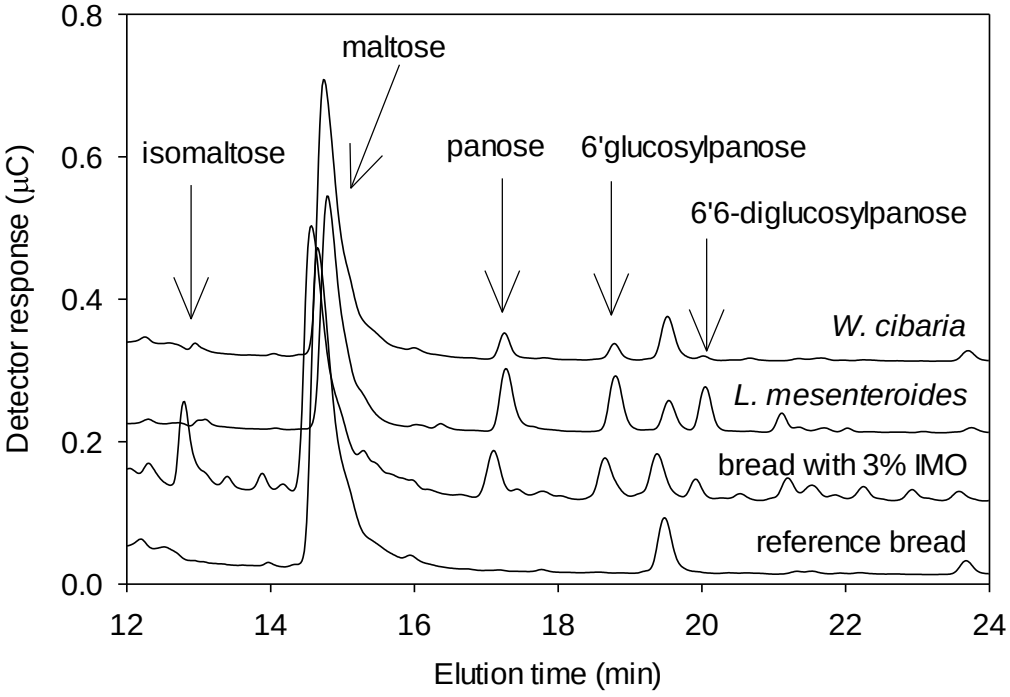

0.8
0.6
0.4
0.2
0.0
Detector response (μC)
maltose
isomaltose
panose
6'glucosylpanose
6'6-diglucosylpanose
W. cibaria
L. mesenteroides
bread with 3% IMO
reference bread
12
14
16
18
20
22
24
Elution time (min)

Figure 4

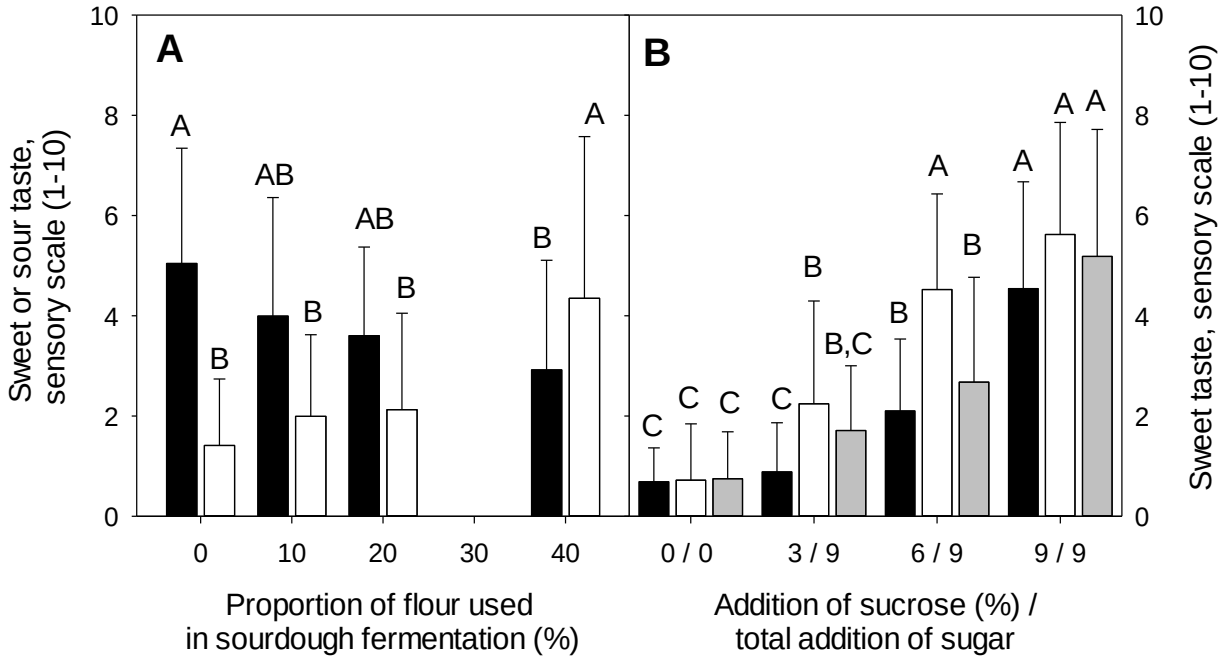

A
B
Sweet or sour taste, sensory scale (1-10)
Sweet taste, sensory scale (1-10)
0
2
4
6
8
10
A
B
AB
B
AB
B
B
A
C
C
C
C
B
B,C
B
A
B
A
A
A
0
10
20
30
40
0 / 0
3 / 9
6 / 9
9 / 9
Proportion of flour used in sourdough fermentation (%)
Addition of sucrose (%) / total addition of sugar

Figure 5.

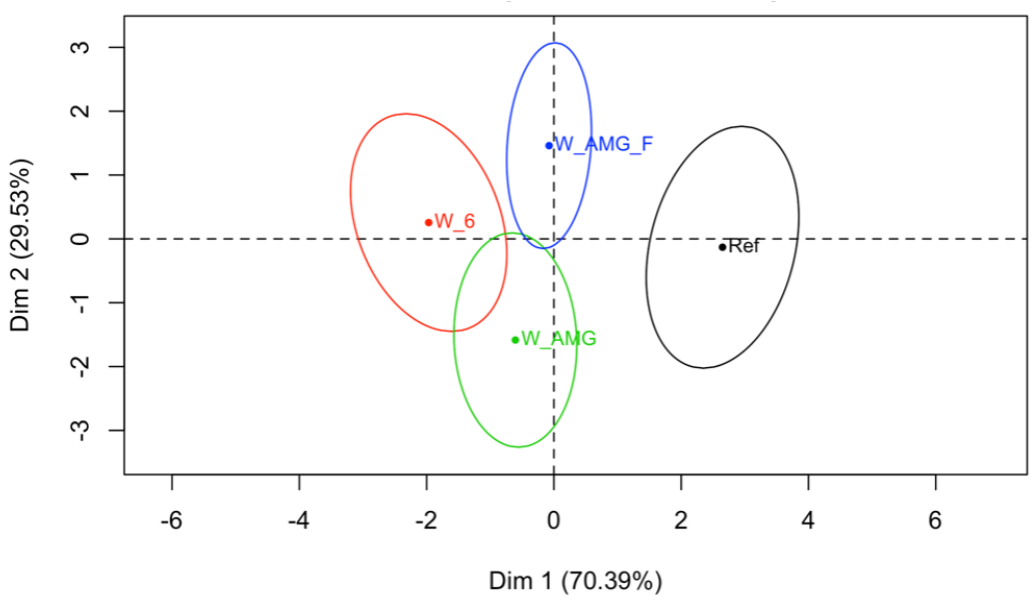


Figure 6.

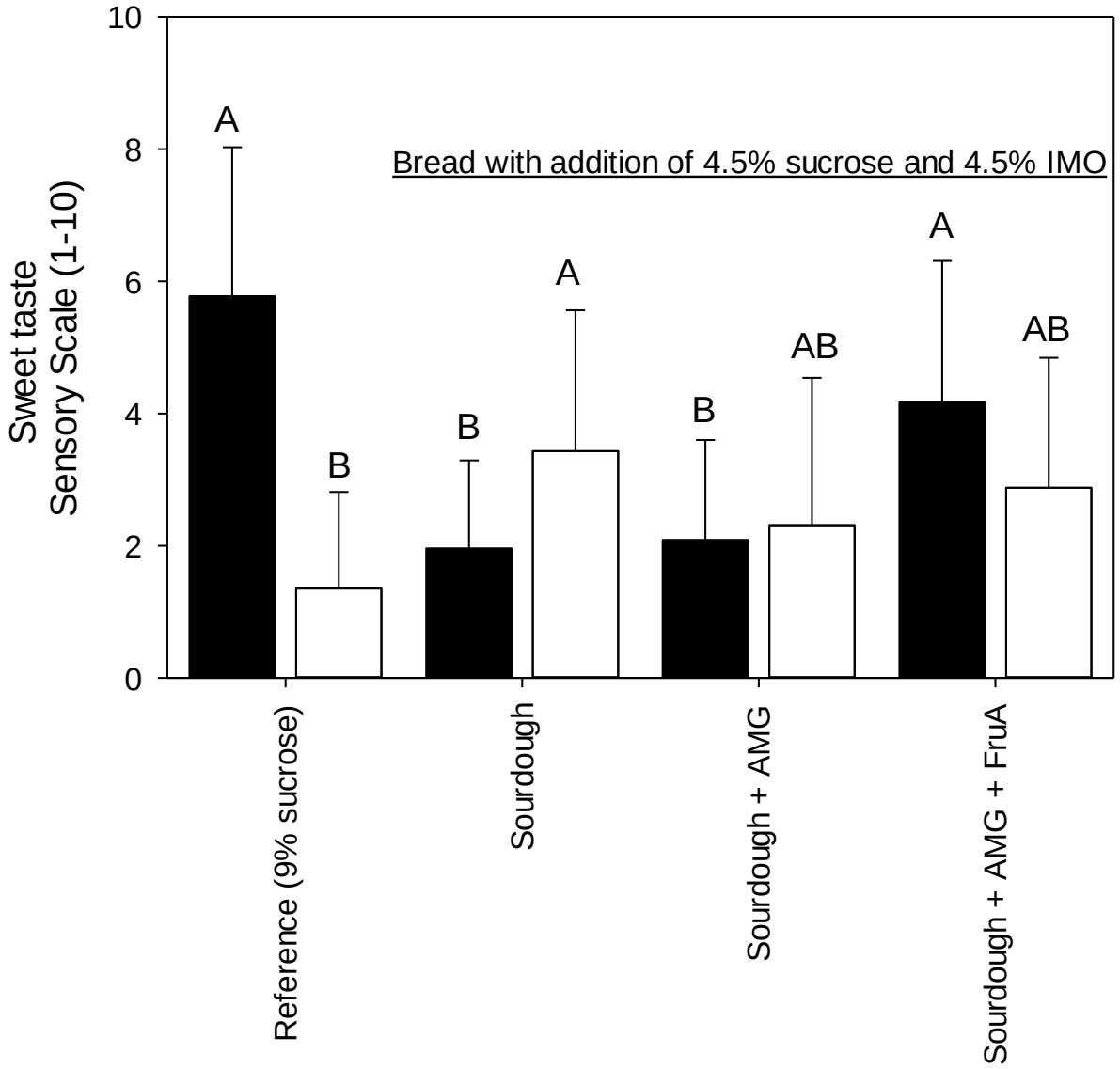
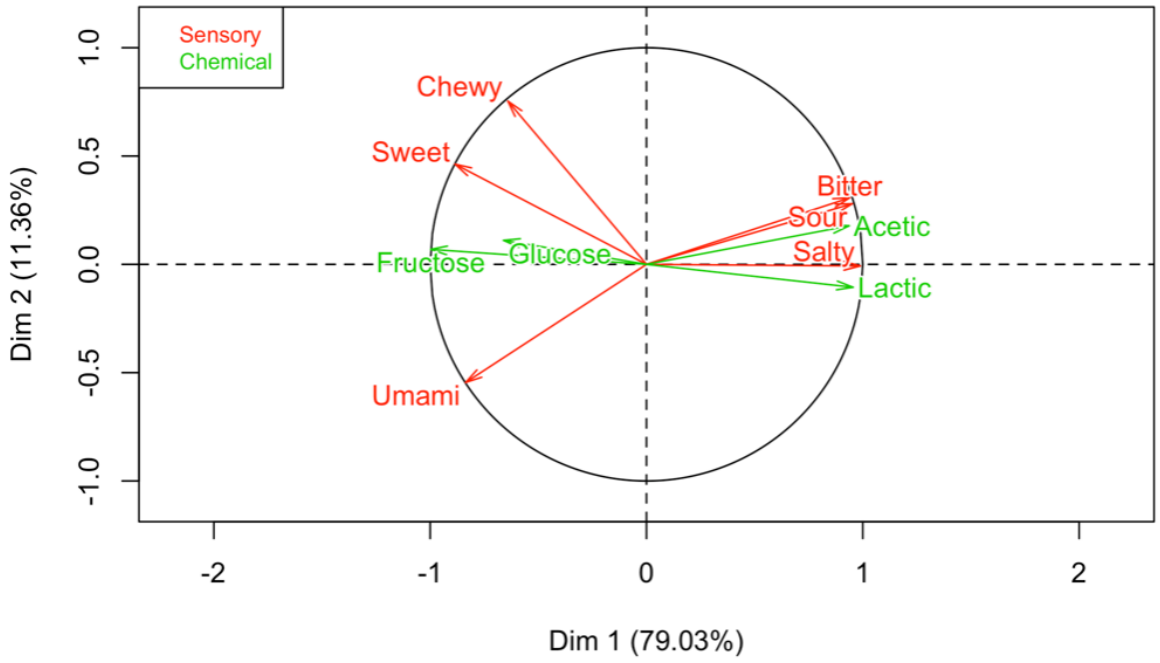

Sweet taste
Sensory Scale (1-10)
10
8
6
4
2
0
Bread with addition of 4.5% sucrose and 4.5% IMO
A
B
B
A
B
AB
A
AB
Reference (9% sucrose)
Sourdough
Sourdough + AMG
Sourdough + AMG + FruA

Figure 7


Sensory
Chemical
Chewy
Sweet
Bitter
Sour
Acetic
Salty
Fructose
Glucose
Lactic
Umami
Dim 2 (11.36%)
Dim 1 (79.03%)
1.0
0.5
0.0
-0.5
-1.0
-2
-1
0
1
2