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The role of dextran and maltosyl-isomalto-oligosaccharides on the structure of bread enriched with surplus bread

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ABSTRACT

Occurrence of surplus bread (SB) is common in the baking industry. Edible surplus bread can be utilized as a new bread dough ingredient; however, it creates technological challenges that affect the quality of the new bread. In this study, the interactions of SB with dough macromolecules were studied in a gluten-starch model dough system and subsequent model bread. Moreover, dextran or maltosyl-isomalto-oligosaccharides (MIMO) were produced by dextransucrase preparation, incorporated into the dough containing SB, and their individual influence on dough rheology and bread structure was investigated. Compared to control model dough/bread, the addition of SB at 10% level significantly decreased extensibility of the dough, dough level, and specific volume (SV) of bread, despite standardized gluten content and optimized water absorption (WA). This confirms that SB constituents (especially gelatinized starch) deteriorate the dough structure-forming by interactions with gluten network. Dextran addition at appropriate level (0.7%) with optimized WA, shielded the gluten network from the interactions of SB, thus, increasing dough extensibility and softness. Furthermore, dextran-enrichment significantly reduced the hardness and staling of breads and increased the SV to the control model bread level. MIMOs, especially at low concentration, induced stronger interactions with gluten proteins than dextran. However, the addition of MIMOs reduced the SV of breads containing SB and did not reduce the overall crumb hardness despite partially preventing starch retrogradation in the early phase of storage. The protective interactions of dextran with dough macromolecules showed that *in vitro* dextran could be utilized to enable recycling of edible SB.

1. Introduction

It is estimated that one third of all produced food is lost or wasted before consumption (Gustavsson, Cederberg, Sonesson, van Otterdijk, & Meybeck, 2011). Enhanced resource efficiency throughout the whole food chain, including prevention of food wastage, is a premise for a sustainable food system. Edible surplus food that is currently downgraded for non-food applications, should be retained for human consumption. Bread is one of the most wasted food product due to its high production volume and short shelf life. The occurrence of surplus bread (SB) is common in bakeries due to over production and/or take-back agreements with the retail market (Brancoli, Bolton, & Eriksson, 2020). Optimally, after minimizing surplus production, edible surplus products should be recycled as new food ingredients. However, recycling SB as a baking ingredient creates technological challenges that affect the quality of the new bread, by reducing the bread volume and increasing the crumb hardness (Goshima et al., 2019; Immonen, Maina, Wang, Coda, & Katina, 2020).

Bread dough is a complex matrix containing a bi-continuous gluten-starch phase and a plasticizing aqueous phase. Macromolecular interactions and entanglements are responsible for the strength and extensibility of the dough which largely determines its bread making performance. Gas-holding capacity and stability of gas cells in the dough eventually determines the homogeneity of bread crumb structure and volume of the bread (Goesaert et al., 2005). The gluten network, and the strain hardening behavior of high-M_w (molecular weight) glutenins, is the primary structure that stabilizes the dough matrix during proofing and baking (Sroan, Bean, & MacRitchie, 2009; van Vliet, 2008). Dough strain hardening resulting from glutenin entanglements has been related to good gas cell stability and overall baking quality (De Bondt, Hermans, Moldenaers, & Courtin, 2021; Dobraszczyk, Smewing, Albertini, Maesmans, & Schofield, 2003). In addition to gluten proteins, the properties of starch have been shown to influence dough strain hardening behavior (McCann et al., 2018). Moreover, the wheat dough structure is supported by a network of water-soluble arabinoxylans and surface-active

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Abbreviations

SB	surplus bread
MIMO	maltosyl-isomalto-oligosaccharides
LAB	lactic acid bacteria
EPS	exopolysaccharide;
M_w	molecular weight
CMB	control model bread
FW	flour weight
WA	water absorption
SHI	strain hardening index
SV	specific volume

compounds which form a stabilizing liquid lamellae in the gas-liquid interface (Courtin & Delcour, 2002; Sroan & MacRitchie, 2009). Bread crumb firmness is an important property associated with staling and consumer acceptance (Gray & Bemiller, 2003). Firming of bread texture is attributed to the rate of starch retrogradation: formation of amylopectin double-helical structures and crystalline regions (Goesaert et al., 2005). However, other phenomena, such as starch-gluten interactions, redistribution of water, and formation of continuous macromolecular structure throughout the crumb are known to influence firming of the bread (Lacaze, Wick, & Cappelle, 2007; Rühmkorf et al., 2012; Zhang et al., 2018).

The negative impact of SB on new bread volume and texture has been associated with dilution of native gluten content in the dough and the gelatinized starch in the SB (Immonen, Maina, Coda, & Katina, 2021). Consequently, enzymatic hydrolysis of bread starch partially reduces the negative effects of SB, which may be utilized to enhance the economic feasibility of the recycling process (Immonen et al., 2021; Rosa-Sibakov et al., 2022). An even more promising way of mitigating the negative impact of bread recycling is tailored fermentation of SB with lactic acid bacteria (LAB) that can produce dextran as was recently evidenced (Immonen et al., 2020).

Dextran, a microbial glucan with α-(1 → 6) backbone and varying amount of α-(1 → 3) branches, is a hydrocolloid and functional compound for clean-label food applications, including improved baking performance, flavor masking, and thickening, among others (Leemhuis et al., 2013; Wang, Maina, Coda, & Katina, 2021). Dextrans are produced by extracellular dextransucrase enzymes (EC.2.4.1.5) of many LAB strains. It is well known that dextran can increase bread volume and decrease crumb hardness in wheat or wheat composite breads and gluten free baking (Galle et al., 2012; Wang et al., 2021). However, the influence of dextran is strongly dependent on molecular features such as linearity and M_w as well as dough formulation and the baking process (Lacaze et al., 2007; Rühmkorf et al., 2012; Zhang et al., 2018). In wheat baking, dextran is known to increase water absorption (WA). Pure high-M_w dextran also enhanced the elasticity of the dough prepared with sourdough (Zhang et al., 2018). However, dextran addition without sourdough did not strongly affect the overall visco-elastic response of the dough (Zannini, Waters, & Arendt, 2014). Dextran has been proposed to enhance the oven rise and retard bread staling (Lacaze et al., 2007; Zhang et al., 2018). The postulated mechanisms behind the anti-staling activity of dextran include suppression of starch gelatinization, improved water retention, and hindering of macromolecular entanglements in the bread, similarly to other hydrocolloids (Fadda, Sanguinetti, Del Caro, Collar, & Piga, 2014; Zannini et al., 2014; Zhang et al., 2018).

In situ production of dextran by fermentation is accompanied with acidification due to LAB metabolism, and production of maltosyl-isomalto-oligosaccharides (MIMO) due to maltose acceptor reactions (Leemhuis et al., 2013). The presence of maltose in the matrix (such as surplus bread), therefore, decreases the dextran yield and leads to the production of a mixture of dextran and a homologous series of MIMO of different molecular weights. Maltose has the highest known affinity for dextransucrase acceptor reaction, but also other mono- and oligosaccharides can act as acceptor molecules (Fu & Robyt, 1990; Hu, Winter, Chen, & Gänzle, 2017). The production of MIMO may be beneficial due to their potential prebiotic properties (Kothari, Patel, & Goyal, 2014; Sorndech, Nakorn, Tongta, & Blennow, 2018), but should be avoided when targeting high dextran yield or pure dextran production. The influence of oligosaccharides on dough rheology and bread staling is dependent on their molecular size distribution. Low-M_w malto-oligosaccharides at low addition levels tend to reduce the elasticity and viscosity of the dough but, in turn, can retard starch retrogradation (Defloor & Delcour, 1999; Miyazaki, Maeda, & Morita, 2004). At present, there is no clear evidence to distinguish the influence of *in situ* produced dextran and MIMO on dough rheology and the textural properties for the final bread. Furthermore, the specific interactions between SB and dough macromolecules (gluten and starch), MIMO and dextrans have not been studied thus far.

The contribution of MIMO to dough structure and bread volume/texture is important to know because the SB composition can be tailored to favor either dextran or MIMO production. Furthermore, based on current literature, it is unclear how experimental dough testing can predict dextran and MIMO functionality for bread making performance.

So far, commercial dextrans have been expensive and only rarely applicable in large scale food production. Isolation of dextransucrase from dextran-producing bacteria has been successfully developed before, and utilized for dextran and MIMO production *in vitro* (Shukla et al., 2014) and in food matrices (da Silva, Rabelo, & Rodrigues, 2014; Kajala et al., 2015). The enzymatic production of dextran with or without MIMO, instead of fermentation, enables high purity and a comparative set up, thus, allowing to specifically study the independent influence of dextran and MIMO (without microbial metabolites) on dough rheology and bread quality.

In our previous studies the vital gluten content has not been standardized in the bread formulation containing SB. The aim of this study was to develop an *in vitro* dextran production by crude enzyme preparation of *Weissella confusa* A16 and study the effects of dextran and MIMO in different addition levels on the model dough extensional and proofing properties, and specific volume, texture, and shelf-life of subsequent model bread. The specific aim was to reveal potential interactions of dextran and MIMO with starch and protein by using a model bread composed only of main components present in bread and with addition of 10% of SB.

2. Materials & methods

2.1. Raw materials

Surplus bread (SB, white wheat bread) was kindly provided from Sinuhe Ky (Lahti, Finland). The bread was ground into <1 mm crumbs, stored at −20 °C before use, and the composition per 100 g was: 46.1 g carbohydrates, 9.3 g protein, 3.2 g fiber, 1.7 g fat and moisture 38.5 g. Before incorporating the SB to the new dough, a bread slurry was prepared by homogenizing SB with RO-water (1:3, w/w) for 1 min using a Bamix blender (Type M 140, 140 W, Switzerland). Gluten flour and wheat starch were purchased from Leipurin Oy (Vantaa, Finland). The composition of gluten flour declared by the manufacturer was per 100 g: fat 5 g, carbohydrates 8.1 g, fiber 1 g, protein 79 g, salt 0.125 g, and for wheat starch the composition was per 100 g: fat 0.1 g, carbohydrates 87 g, fiber 0 g, protein 0.2 g, salt 0.05 g. The moisture content of gluten flour was 5,49% and wheat starch 11.49%, which were measured using the AACC 45–15.02 gravimetric method.

2.2. Production of dextransucrase of Weissella confusa A16

Weissella confusa A16 (Wang et al., 2019) strain was maintained in

MRS broth and cultivated in MRS+2% sucrose broth for 24 h at 30 °C before use. 200 μl of the culture was transferred into a 10 ml enzyme production medium (EPM) containing per 100 ml: sucrose 2 g, maltose 1 g, yeast extract 2 g, K_2HPO_4 2 g, $MgSO_4$ x $7H_2O$ 0.02 g, $MnSO_4$ x $4H_2O$ 0.001 g, $FeSO_4$ x $7H_2O$ 0.001 g, $CaCl_2$ x $2H_2O$ 0.001 g and NaCl 0.001 g. The pH of the medium was adjusted to 6.9 with 4 M HCl and the culture incubated at 25 °C for 16 h. The cell culture, after centrifugation (10 000×*g* at 20 °C for 15 min) from 10 ml EPM, was inoculated to a new 100 ml EPM and incubated at 25 °C for 6 h. The culture was then divided into sterile Falcon tubes and centrifuged (10 000×*g* at 4 °C for 20 min), and the cell-free supernatant containing dextransucrase was collected and stored at 5 °C. The enzyme preparation was used within three weeks during which time the enzyme activity remained over 80% of the initial activity.

2.3. Enzyme activity assay

Dextransucrase activity was measured as the release of fructose in given conditions (modified from Shukla et al. (2014)). The activity assay of dextransucrase was conducted in a 1 ml mixture of 900 μL reaction solution, either MilliQ water or buffer (20 mM sodium acetate buffer pH 5.4) containing 5% sucrose and 0.3 mM $CaCl_2$, and 100 μL enzyme preparation. The reaction mixture was incubated in a 35 °C water bath for 30 min. Immediately after incubation, the samples were centrifuged at 13 000 rpm for 10 min through Amicon 0.22 μm filters (10kDA cut-off, Merck Millipore Ltd, Cork, IR) to remove the enzymes. Free sugar composition was analyzed by HPAEC-PAD as described in section 2.5. below. The enzyme activity was reported as units (U/ml) in the enzyme preparation, one U defined as the release of 1 μmol of fructose per min at 35 °C (Kim, Robyt, Lee, Lee, & Kim, 2003; Shukla et al., 2014).

2.4. In vitro production of dextran and maltosyl-isomalto-oligosaccharides

Dextran and MIMO were produced in a water solution containing 0.3 mM $CaCl_2$, 10% (v/v) dextransucrase preparation, and either 10% sucrose (w/v, later referred as DEX-solution) only, or 10% sucrose + 2.5% maltose (later referred as MIMO-solution, sucrose from Dan Sukker, Finland, maltose from Sigma-Aldrich, St. louis, USA). Sugars and $CaCl_2$ were solubilized in RO-water followed by the addition of the dextransucrase preparation. Finally, the volume was adjusted with RO-water, manually mixed, and the mixture was statically incubated for 48 h at 35 °C. After that the dextran and MIMO solutions were stored at 5 °C and used within three weeks. The dextran or MIMO solutions were carefully manually mixed with SB slurry 15 min prior to use for model dough tests and model bread preparation.

2.5. Quantification of carbohydrate profile

The carbohydrate profile of dextran and MIMO solutions were measured. For free sugar and oligosaccharide analysis, the samples were diluted with MilliQ-water and centrifuged through Amicon 0.22 μm filters. Samples were analyzed for free sugars according to Xu et al. (2017) using anion exchange chromatography (HPAEC-PAD) equipped with a CarboPac PA1 column (Dionex, Sunnyvale,CA,USA). The oligosaccharide profile was analyzed according to Immonen et al. (2021) using HPAEC-PAD equipped with a CarboPac PA100 column (Dionex). Xylotriose (Megazyme, Ireland) was used as an internal standard and panose (TCI Europe nv, Belgium) was used to prepare a standard curve (0.01–0.5 mg/ml, $R^2 > 0.99$). Due to lack of appropriate MIMO standards, the overall content of MIMO was approximated by calculating the sum of MIMO peak heights compared to that of panose. The MIMO concentration was thus reported as panose equivalents. Dextran was analyzed with enzyme-assisted method according to Katina et al. (2009) followed by glucose quantification using HPAEC-PAD similarly to free sugars described above.

2.6. Starch-gluten model dough testing

2.6.1. Dough formulation

The model dough formulations were prepared according to the recipes in Table 1. Starch and gluten (86/14 w/w) were always pre-mixed before mixing with other ingredients, forming a flour base containing 11.23% protein. The fructose content in the dough was 2.8% flour weight (FW) level as a substrate for yeast leavening instead of sucrose. Therefore, free fructose in dextran and MIMO solutions replaced part of the fructose in the recipe. Furthermore, the 10% SB addition replaced an equal amount of starch (dry matter basis) thus maintaining the constant native gluten content. Three levels of a DEX-solution (DEX 1–3) or MIMO-solution (MIMO 1–3) was added to the dough formulation, partly replacing water and fructose in the dough. Dextran and MIMO themselves were additional substances, thus not replacing anything in the dough formulation. The optimal WA was defined as described below in the Farinograph part.

2.6.2. Farinograph

The model dough WA was determined using a Farinograph-E (Brabender, Germany) with a 300 g mixing bowl (AACC 54–21.02 method). Based on pre-trials including a baking test, the model dough consistency in the Farinograph (aiming at 500 FU) did not produce optimal consistency for baking similarly to wheat dough. It was found that the consistency of 360 FU at 5 min mixing produced a dough with maximal WA, yet not too sticky to handle. The pre-trials also confirmed that the consistency of 360 FU at 5 min was the optimal for doughs containing SB, and SB with dextran. Therefore, the WA of all doughs was adjusted so that the consistency reached 360 ± 10 FU after 5 min of mixing.

2.6.3. Uni-axial extensional test

The uni-axial extensional test was performed using a Kieffer gluten extensibility rig mounted on a texture analyzer (TA-XT2i, Stable Micro Systems Ltd., UK) according to Wang et al. (2018). Doughs for the uni-axial test were prepared using 200 g flour basis and excluding yeast and salt from the recipe according to Table 1. The dough was mixed for 5 min in the Farinograph to reach 360 FU consistency, rested for 20 min (35 °C, 75% relative humidity), molded, and rested for a further 40 min (35 °C, 75% relative humidity). Each dough type was prepared in duplicate and at least eight replicate dough stripes from each dough were measured for maximum resistance to extension (g) and extensibility (mm).

2.6.4. Bi-axial extensional test

The bi-axial extensional test was performed using a D/R Dough Inflation System mounted on a texture analyzer (TA.XTPlus 100, Stable Micro Systems Ltd.). Doughs were prepared using 300 g flour basis according to Table 1 (excluding yeast and salt), mixed 5 min in Farinograph to 360 FU consistency, bench-rested for 5 min, manually sheeted to approximately 8 mm thickness, cut into spheres with a 55 mm diameter using a round cutter, and pressed to 2.67 mm height within a sample retainer using a height-adjusted manual press. The samples were rested in the retainers for 10 min in room temperature (22 ± 1 °C) before the bi-axial extensional test. The dough inflation test was carried out using 40 cm^3 trigger volume and 26.7 cm^3 flow rate until a bubble failure was recorded (detected as pressure drop below 0.5 inch of water), providing a pressure-drum distance curve. Each dough was prepared in duplicate and five replicate dough sheets were measured. Peak pressure (mm), maximum extension (drum distance, mm), bubble burst strain (Hencky strain at burst point) and strain hardening index (curve fitting) were automatically calculated by the exponent program (Stable Micro Systems).

Table 1
Starch-gluten model dough recipes presented as ingredient weights and percentages in flour weight (FW) basis.

Dough/Bread Type			Gluten	Starch	Water[c]	Surplus Bread	DEX/MIMO-solution[a]	Salt	Fructose[b]	Yeast	Total
Control Dough/Bread		Weight (g)	133.0	817.0	536.8	–	–	14.3	26.6	47.5	1575.1
		% FW	14.0	86.0	56.5	–	–	1.5	2.8	5.0	165.8
10% Surplus Bread	10% SB	Weight (g)	133.0	751.0	555.2	95.0	–	14.3	26.6	47.5	1622.6
		% FW	14.0	79.1	58.4	10.0	–	1.5	2.8	5.0	170.8
	DEX 1	Weight (g)	133.0	751.0	458.6	95.0	149.7	14.3	20.3	47.5	1669.3
		% FW	14.0	79.1	48.3	10.0	15.8	1.5	2.1	5.0	175.7
	DEX 2	Weight (g)	133.0	751.0	376.5	95.0	261.9	14.3	15.6	47.5	1694.8
		% FW	14.0	79.1	39.6	10.0	27.6	1.5	1.6	5.0	178.4
	DEX 3	Weight (g)	133.0	751.0	308.8	95.0	374.1	14.3	10.9	47.5	1734.5
		% FW	14.0	79.1	32.5	10.0	39.4	1.5	1.1	5.0	182.6
	MIMO 1	Weight (g)	133.0	751.0	438.6	95.0	149.7	14.3	20.3	47.5	1649.3
		% FW	14.0	79.1	46.2	10.0	15.8	1.5	2.1	5.0	173.6
	MIMO 2	Weight (g)	133.0	751.0	345.1	95.0	261.9	14.3	15.6	47.5	1663.3
		% FW	14.0	79.1	36.3	10.0	27.6	1.5	1.6	5.0	175.1
	MIMO 3	Weight (g)	133.0	751.0	251.6	95.0	374.1	14.3	10.9	47.5	1677.4
		% FW	14.0	79.1	26.5	10.0	39.4	1.5	1.1	5.0	176.6

[c] Water amount in the dough was optimized, as described in section 2.6.2., and balanced considering the moisture occurring in starch, surplus bread and DEX or MIMO solutions.
[a] Dextran solution was added to DEX-doughs and maltosyl-isomalto-oligosaccharide solution to MIMO-doughs.
[b] Fructose addition amount to the dough was balanced considering the sugars present in DEX and MIMO-solutions.

2.6.5. Maturograph

The doughs for maturograph were prepared according to Table 1 using 200 g flour basis. The doughs were mixed in the Farinograph for 5 min to 360 FU consistency, rested, divided, shaped, and measured exactly as described by Immonen et al. (2021) using Maturograph type 870 103 (Brabender). Each dough type was prepared in duplicate, and the dough level (MU, Maturograph units), dough elasticity (MU) and final proofing period (min) were recorded manually from the obtained maturograms.

2.7. Model bread preparation

Model breads were formulated according to recipes described in Table 1. Starch and gluten were manually pre-mixed thoroughly and water (RO), salt (Meira, Helsinki, Finland), fructose (Suomen Sokeri Oy, Kantvik, Finland) and yeast (Suomen Hiiva Oy, Rajamäki, Finland) were blended in. Additionally, the SB slurry or slurry containing a dextran or MIMO solution were added to their respective bread types. The dough was mixed 2 min slow +3 min fast with a spiral mixer (Diosna Dierks & Söhne GmbH, Germany) and the dough temperature was controlled to reach 25 ± 1 °C immediately after mixing. The dough was then rested, divided, molded, proofed, and baked exactly as described by Immonen et al. (2020). One hour after baking, the breads were weighed, packed, and sealed in plastic bags and stored at room temperature (22 ± 1 °C). All model bread types were prepared in duplicate doughs (triplicate for control model bread, CMB) and each dough produced six replicate breads.

2.8. Specific volume and texture of bread

Dough yield was reported as a sum of dough ingredient amounts (%/FW) and bread yield as dough yield minus weight loss during baking and cooling. Bread volume was measured from three replicate breads from each dough using a BreadVolScan laser scanner (Backaldrin, Austria). The measurement was done on day 1 after baking and the specific volume (SV, g/ml) was calculated as loaf weight/loaf volume. Model bread crumb texture profile was measured on day 1 and day 4 after baking using a texture analyzer (TA-XT2i) equipped with a 5 kg load cell and a P/36R cylindrical probe. For texture profile analysis nine standard size (2.5 × 2.5 cm) crumb cubes were carefully cut from the center of three bread loaves and measured immediately using modified AACC 74-09 method with 40% compression strain, 2.0 mm/s test speed, and 1.0 g trigger force. The staling rate (g/day) was calculated for each bread type as the increase of hardness between day 1 & day 4.

2.9. Differential scanning calorimetry

The amylopectin retrogradation in the model breads was measured on day 1 and day 4 after baking using differential scanning calorimeter DSC823e (Mettler-Toledo Inc., Switzerland) according to Wang et al. (2019). Six replicate crumb pieces from each bread type were heated in sealed aluminum pans in the DSC chamber from 25 °C to 100 °C, 5 °C/min, and onset (T_o), peak (T_p) and endset (T_e conclusion) temperatures were recorded. The enthalpy change (ΔH, J/g), describing the amylopectin melting enthalpy, was calculated as the integrated area between T_o and T_e, automatically normalized with the sample weight.

2.10. Statistical analysis

The results were compared by one-way analysis of variance (ANOVA) and Tukey's post hoc test (p-value < 0.05) using SPSS Statistics 25 software (IBM Corp., NY, USA). A Pearson's correlation analysis (SPSS, 2-tailed, p-value < 0.05 or 0.01) was performed for the dough testing parameters in relation to the key bread quality measures.

3. Results

3.1. Production of dextran and maltosyl-isomalto-oligosaccharides

Weissella confusa A16 was used to obtain a crude dextransucrase preparation. The cell-free dextransucrase preparation contained a small amount of residual dextran and *W. confusa* A16 metabolites. Dextran only or and MIMO were synthetized *in vitro* using the enzyme preparation with water and sucrose (DEX-solution) or water, sucrose, and maltose (MIMO-solution). The measured enzyme activity of the preparation was 7.94 ± 0.2 U/ml in a buffer solution and 7.86 ± 0.29 U/ml in a water solution. Therefore, the DEX/MIMO production solution contained 0.786U/ml dextransucrase and 100 mg/ml (292 mM) sucrose. The addition of maltose (25 mg/ml, 73 mM) to the solution drove the production of MIMO instead of dextran, simultaneously speeding up the rate of sucrose consumption and fructose release (Table 2). Based on preliminary enzyme activity trials, the initial dextransucrase activity was 12% higher in the solution containing 5% sucrose and 1.25% maltose, than in the solution with 5% sucrose only.

By manual assessing, the DEX-solution was very viscous, but the MIMO-solution was only slightly viscous. The carbohydrate profiles of solutions are presented in Table 2. After the incubation (48 h, 35 °C), the DEX-solution contained 25.5 mg/g dextran and 26.6 mg/g fructose, with 27.2 mg/g sucrose remaining. The MIMO-solution, in turn, contained

Table 2
Carbohydrate profiles of DEX-solution and MIMO-solution, presented as average ± standard deviation.

	mg/g				
	sucrose	fructose	maltose	MIMO[a]	dextran
DEX-solution	27.2 ± 2.5	26.6 ± 0.4	–	–	25.5 ± 2.8
MIMO-solution	–	46.7 ± 0.7	2.1 ± 0.2	51.8 ± 0.5	9.1 ± 0.6

[a] As panose equivalents.

9.1 mg/g dextran, 46.7 mg/g fructose and approximately 52 mg/g MIMOs (panose equivalent). A small amount of maltose remained but all the sucrose was consumed during MIMO production. Based on HPAEC-PAD analysis, a homologous series of at least 12 MIMO were present in the solution containing sucrose and maltose (Fig. 1), but no MIMO formation occurred in the solution with sucrose alone.

3.2. Water absorption, rheological and proofing properties of dough

The gluten-starch model dough experiment was carried out according to the recipes shown in Table 1 and the results are presented in Table 3. Based on the measured carbohydrate content in the DEX/MIMO solutions, dextran levels in the doughs were approximately 0.4, 0.7 and 1.0%/FW (DEX 1, 2 & 3, respectively), and MIMO levels 0.8, 1.4 and 2.0%/FW (MIMO 1, 2 & 3, respectively). However, the doughs with added MIMO-solution contained also 0.14, 0.25 and 0.36%/FW residual dextran that was produced concurrently with MIMOs.

The dough WA was defined by Farinograph consistency measurement targeting at 360 FU at 5 min mixing, based on preliminary baking tests (data not shown) producing bread with the highest specific volume (SV). SB addition to the dough increased the optimal WA from 56.5 to 61.5%, and dextran further increased the WA up to 65.5–71% range, thus leading to higher dough yield. The addition of MIMO-solution increased dough WA to the range of 63–64%. Even though the Farinograph consistency of all the doughs was the same at the end of mixing, significant rheological differences were observed among dough types.

Compared to the control, the 10% addition of SB increased the bi-axial peak pressure (P, tenacity) by 36% and reduced the drum distance (L, bi-axial extensibility) by 31%, thus also increasing the P/L ratio from 2.3 to 4.5. Moreover, the bubble burst strain was decreased by 15% due to the addition of SB, but the strain hardening index was not significantly affected. In the uni-axial extensional test, resistance to extension (R) was reduced by 13% and extensibility (E) by 17% due to SB, maintaining the E/R ratio comparable control model dough. The Maturograph test showed that the addition of SB decreased dough level in the same ratio as E (−17%). However, the final proofing period and elasticity were not affected.

Additional rheological changes were observed when dextran or MIMO were incorporated to model doughs containing 10% SB. The addition of dextran at three levels gradually decreased the bubble burst strain and uni-axial R. Meanwhile, bi-axial P remained unchanged, but L decreased by up to 26%, compared to addition of SB only, thus also increasing the P/L ratio to 5.5–5.7 level. Only the addition level of DEX 2 (0.7%/FW) maintained the strain hardening index and increased uni-axial E the most. Lower and higher dextran addition levels decreased the strain hardening index; however, DEX 1 (0.4%/FW) had no significant effect. The uni-axial test showed that dextran gradually decreased the R by up to 30% and increased R/E ratio by up to 47% compared to SB only. The impact of dextran additions on Maturograph proofing parameters were not statistically significant. However, the lowest dextran addition level produced the highest dough level which was 6% higher compared to SB only.

MIMOs increased the bi-axial P by 11–23% and reduced L by 13–23%, thus increasing the P/L ratio to 5.6–6.7 level. Moreover, the MIMOs decreased the bubble burst strain and the strain hardening index by 6–11 and 8–13%, respectively, compared to SB only. In the uni-axial test, a clear dose-response trend was observed with MIMO addition levels. The increasing MIMO level reduced the R and the E, thus increasing the E/R ratio. The dough containing 1.4% MIMOs (MIMO 2) showed identical uni-axial extensional properties to the dough containing SB only. Maturograph test showed that MIMOs did not influence

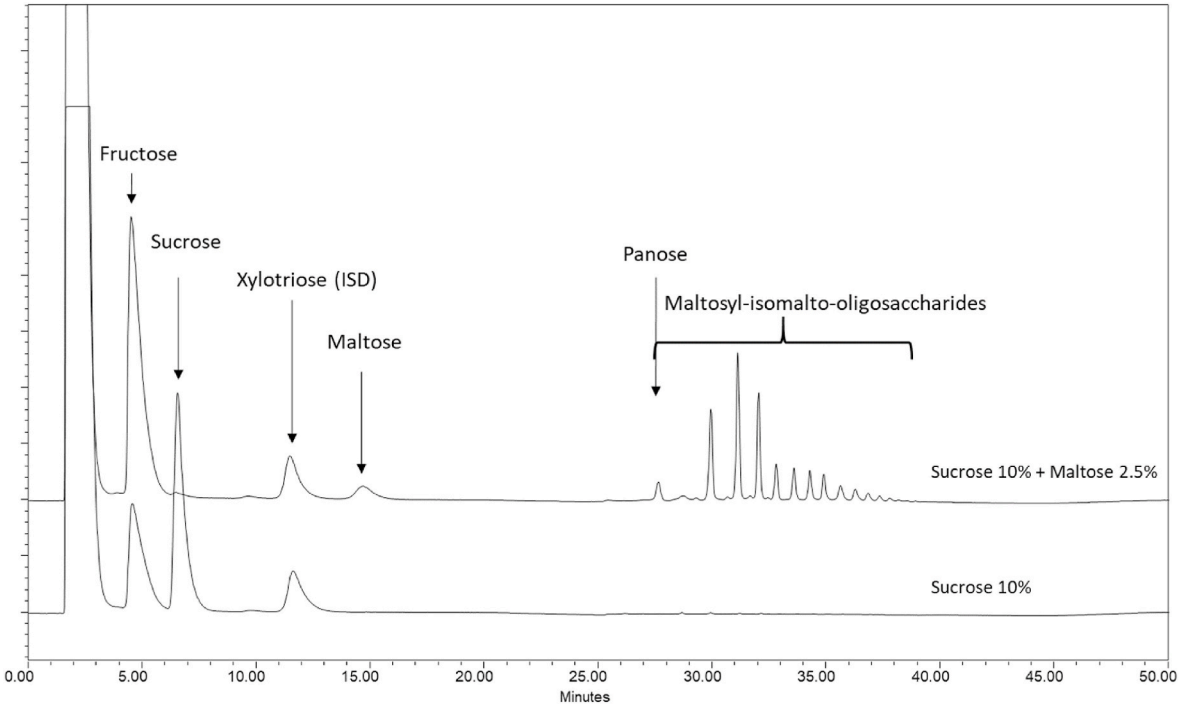

Fig. 1. HPAEC-PAD chromatograms of DEX-solution (10% sucrose) and MIMO-solution (10% sucrose + 2.5% maltose) incubated (48 h, 35 °C) with W. confusa A16 dextransucrase preparation.

Table 3
Dough rheological parameters, presented as average ± standard deviation. Different letter within the same row indicates statistical difference at $P < 0.05$ level.

Test Type	Parameter	Control Model Dough	10% Surplus Bread						
			10% SB	DEX 1	DEX 2	DEX 3	MIMO 1	MIMO 2	MIMO 3
Farinograph	Optimal WA[b] (%/FW)	56.5	61.5	65.5	67.5	71.0	63.0	63.5	64.0
Bi-axial extensional test	Peak Pressure P (mm)	99 ± 15a	135 ± 16b	139 ± 19b	130 ± 10b	129 ± 11b	166 ± 17c	161 ± 9c	150 ± 21bc
	Drum Distance L (mm)	45 ± 9a	31 ± 5b	26 ± 2bc	23 ± 2c	23 ± 2c	26 ± 3bc	24 ± 2c	27 ± 4bc
	P/L Ratio	2.3 ± 0.6a	4.5 ± 1.0b	5.5 ± 1.2bc	5.6 ± 0.5bc	5.7 ± 0.8bc	6.6 ± 1.1c	6.7 ± 0.4c	5.6 ± 0.9bc
	Bubble Burst Strain (Hencky)	1.86 ± 0.14a	1.59 ± 0.11b	1.46 ± 0.07bc	1.39 ± 0.07c	1.37 ± 0.09c	1.45 ± 0.07c	1.41 ± 0.07c	1.49 ± 0.10bc
	Strain Hardening Index	1.46 ± 0.06a	1.42 ± 0.05 ab	1.34 ± 0.06bc	1.41 ± 0.04 ab	1.27 ± 0.09cd	1.31 ± 0.06cd	1.24 ± 0.05d	1.26 ± 0.08cd
Uni-axial extensional test	Res. To Extension R (g)	20.1 ± 1.9a	17.5 ± 2.6b	15.0 ± 1.2c	13.3 ± 0.8cd	12.3 ± 1.5d	19.1 ± 3.1 ab	17.8 ± 2.8b	14.9 ± 1.7c
	Extensibility E (mm)	13.8 ± 1.9a	11.5 ± 1.3bc	12.5 ± 1.5abc	13.0 ± 1.6 ab	11.5 ± 1.8bc	12.0 ± 1.8bc	11.6 ± 1.5bc	11.3 ± 1.1c
	E/R Ratio[a]	0.68	0.66	0.83	0.97	0.94	0.63	0.66	0.75
Maturograph	Dough Level (MU)	740 ± 18a	614 ± 35bc	653 ± 49b	623 ± 22bc	603 ± 35bc	626 ± 16bc	580 ± 26cd	536 ± 26c
	Final Proofing Period (min)	59 ± 5a	59 ± 5a	60 ± 7a	56 ± 4a	60 ± 7a	61 ± 8a	59 ± 7a	62 ± 6a
	Dough Elasticity (MU)	263 ± 21a	259 ± 11a	265 ± 22a	269 ± 16a	261 ± 14a	260 ± 16a	258 ± 26a	255 ± 12a

[b] Optimal water absorption, defined as the % FW water required to reach model dough consistency of 360 FU at 5 min mix.
[a] Extensibility/Resistance to extension ratio calculated from dough result averages.

the final proofing period or elasticity (similarly to dextran), however, the MIMO 2 and 3 additions (1.4 and 2.0%/FW) reduced the dough level by 6% and 13%, respectively.

3.3. Specific volume and shelf-life of model bread

The images of all types of model bread, whole loaves and identically cut pieces, are presented in Fig. 2. Model dough formulations used in bread making are presented in Table 1, and the bread quality measurement results are presented in Table 4. The addition of SB increased the dough yield by 3% and the bread yield by 3.6% due to increased dough WA and slightly reduce weight loss during baking and cooling. Moreover, SB reduced the SV of bread by 12.5% compared to control model bread (CMB), and produced breads with darker crusts (visually assessed, Fig. 2). Bread crumb hardness on day 1 and 4 was not significantly affected by SB (Fig. 3), but the staling rate was reduced from 61 g/day (in the CMB) to 51 g/day. Additionally, breads containing SB had 14% higher resilience of the crumb on day 1, but on day 4 the resilience was decreased to the same level of CMB. Bread crumb springiness was not affected by SB. According to the DSC measurements, the melting enthalpy of retrograded amylopectin in the bread containing SB was 11% higher on day 1 but 4% lower on day 4 compared to CMB. The

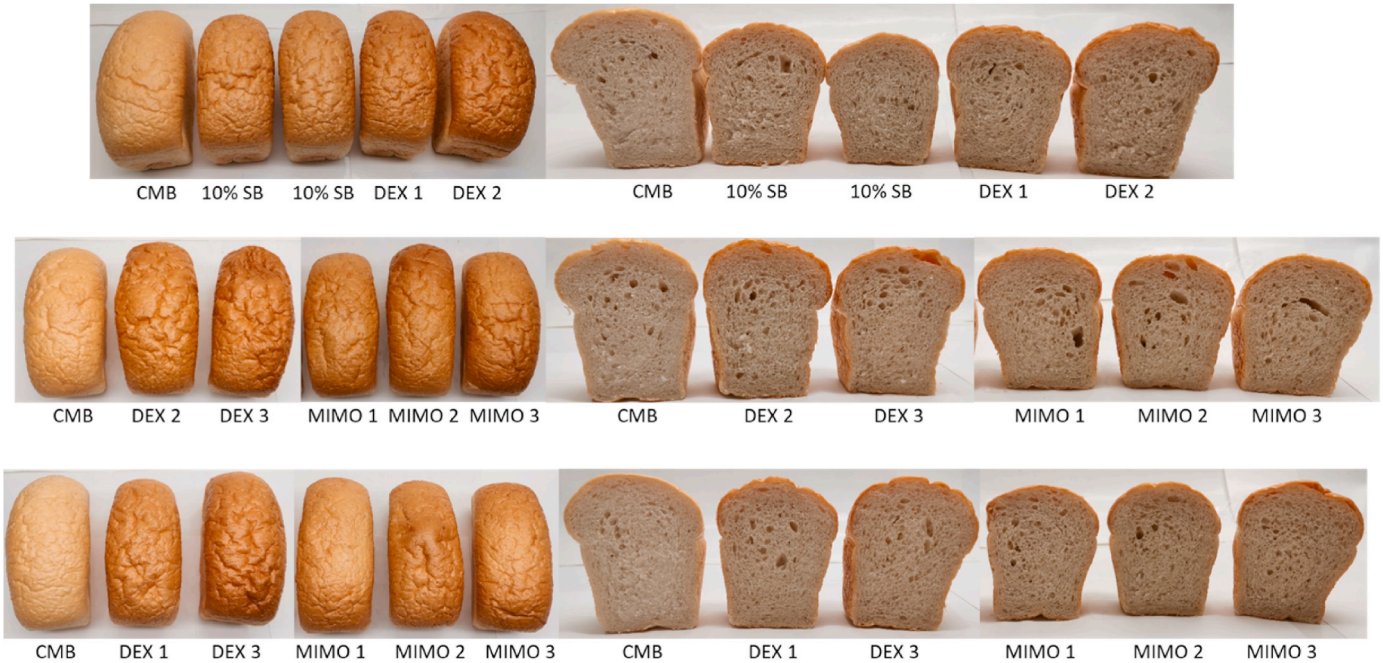

Fig. 2. (Colored figure). Photos of breads on day 1 after baking. CMB = control model bread, 10% SB = bread with 10% surplus bread, DEX 1–3 = breads with 10% surplus bread and 0.4, 0.7 and 1.0% dextran, respectively, MIMO 1–3 = breads with 10% surplus bread and 0.8, 1.4, and 2.0% MIMO, respectively.

Table 4
Bread quality parameters, presented as average ± standard deviation. Different letter within the same row indicates statistical difference at $P < 0.05$ level.

Parameter		Unit	Control Model Bread	10% Surplus Bread						
				10% SB	DEX 1	DEX 2	DEX 3	MIMO 1	MIMO 2	MIMO 3
Dough Yield		%/FW	165.8	170.8	175.7	178.4	182.6	173.6	175.1	176.6
Weight loss		%	13.3 ± 0.4 ab	12.8 ± 0.3c	13.2 ± 0.2b	13.5 ± 0.2a	13.6 ± 0.3a	12.7 ± 0.2c	12.6 ± 0.3c	12.5 ± 0.2c
Bread Yield[b]		%/FW	143.8	149.0	152.6	154.3	157.8	151.5	153.0	154.4
Bread Dry Matter		%	73.2	71.0	70.3	70.1	69.3	70.8	70.7	70.7
Specific Volume		ml/g	4.8 ± 0.2a	4.2 ± 0.1 cd	4.3 ± 0.1bc	4.7 ± 0.1a	4.4 ± 0.1b	4.1 ± 0.2de	4.0 ± 0.1ef	3.9 ± 0.1f
Resilience	Day 1		0.35 ± 0.2a	0.40 ± 0.2b	0.41 ± 0.2bc	0.40 ± 0.2bc	0.42 ± 0.2c	0.39 ± 0.2b	0.39 ± 0.2b	0.39 ± 0.2b
	Day 4		0.26 ± 0.2a	0.27 ± 0.2 ab	0.29 ± 0.2b	0.29 ± 0.2bc	0.30 ± 0.2c	0.27 ± 0.2 ab	0.27 ± 0.2a	0.26 ± 0.2a
Staling Rate[a]		g/day	61	51	49	46	43	55	58	56
DSC Day 1	T_o	°C	42.8 ± 0.6a	42.9 ± 0.4a	44.2 ± 0.7b	44.1 ± 0.9b	44.4 ± 0.5bc	45.2 ± 0.3cd	45.4 ± 0.3d	45.5 ± 0.1d
	T_p		57.6 ± 0.4 ab	57.2 ± 0.4 ab	57.4 ± 0.3 ab	56.9 ± 0.5a	57.3 ± 0.6 ab	57.6 ± 0.4 ab	57.9 ± 0.3b	57.5 ± 0.2 ab
	T_e		73.5 ± 0.7a	72.8 ± 0.6 ab	71.9 ± 0.3bc	71.5 ± 1.1c	71.6 ± 0.9bc	71.8 ± 0.2bc	72.0 ± 0.5bc	72.1 ± 0.6bc
	ΔH	J/g	1.28 ± 0.22 ab	1.42 ± 0.17b	1.23 ± 0.11 ab	1.27 ± 0.11 ab	1.14 ± 0.05a	1.09 ± 0.09a	1.11 ± 0.08a	1.17 ± 0.10a
DSC Day 4	T_o	°C	45.2 ± 0.8 ab	44.8 ± 1.0b	45.1 ± 1.1 ab	46.0 ± 0.3a	44.9 ± 0.4 ab	44.7 ± 0.2b	44.7 ± 0.2b	44.9 ± 0.3 ab
	T_p		57.6 ± 0.8a	57.3 ± 0.5 ab	56.9 ± 0.7 ab	57.0 ± 0.4 ab	56.4 ± 0.3b	56.8 ± 0.1 ab	56.5 ± 0.3b	56.7 ± 0.5 ab
	T_e		73.6 ± 0.8a	72.1 ± 0.4b	71.1 ± 0.8c	70.9 ± 0.4c	70.4 ± 0.4c	71.3 ± 0.4bc	71.0 ± 0.5c	71.4 ± 0.3bc
	ΔH	J/g	2.05 ± 0.15 ab	1.94 ± 0.21 ab	2.13 ± 0.17b	1.80 ± 0.09a	2.02 ± 0.17 ab	1.98 ± 0.03 ab	1.98 ± 0.07 ab	2.02 ± 0.07 ab

[b] Theoretically calculated as the amount of bread that can be produced with that dough formulation and measured weight loss during baking and cooling.
[a] Calculated from hardness result averages (Fig. 2).

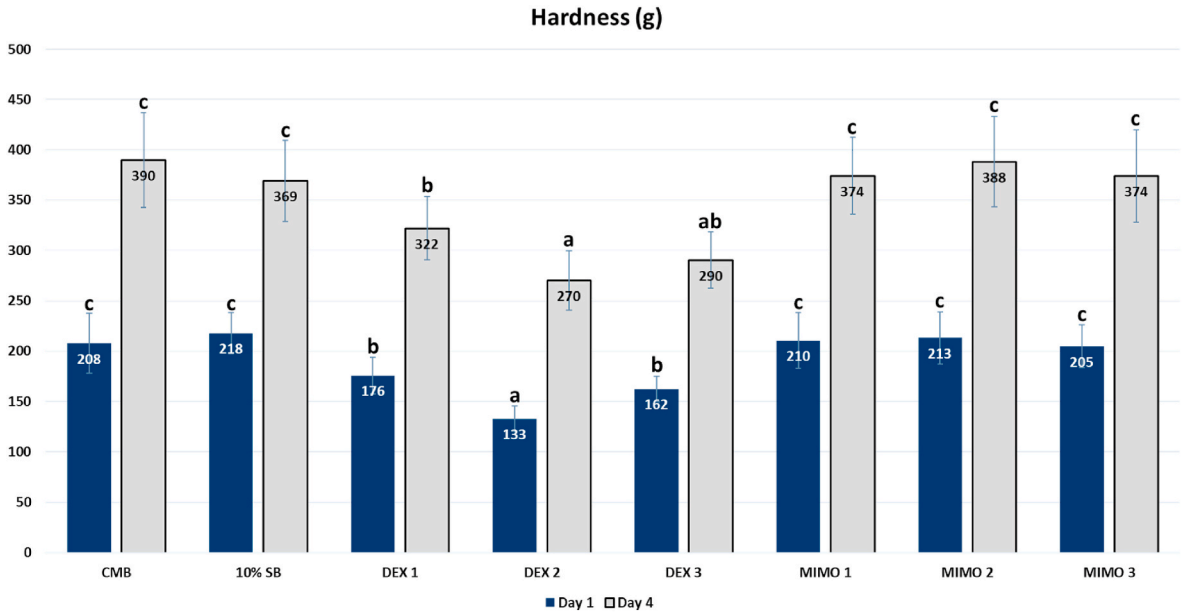

Fig. 3. (Colored figure). Bread crumb hardness results from different bread types on days 1 and 4. CMB = control model bread, SB = bread with 10% surplus bread, DEX = breads with 10% surplus bread and added dextran solution (3 levels), MIMO = breads with 10% surplus bread and added maltosyl-isomalto-oligosaccharide solution (3 levels). Different letter within the same row indicates statistical difference at $P < 0.05$ level.

onset, peak, and endset temperatures remained unchanged.

Visually assessing, dextran- and MIMO-enrichment mildly darkened the crust of bread but no clear changes in crumb structure were observed. Dextran incorporation to the breads containing SB increased the dough yield by 3–7% due to higher WA per addition level. Weight loss during baking and cooling was further increased by 3–6%, which is attributed to higher loaf volume (more evaporation surface) and/or high moisture content in the dough. Consequently, the bread yield was increased by 2–6%. All three levels of dextran addition increased the SV of bread. The addition level of 0.7% (DEX 2) increased SV the most (12%), up to the level of CMB. Dextran levels 0.4% and 1.0% increased the SV by 2.4 and 4.8%, respectively. Furthermore, dextran influenced bread texture by decreasing hardness and staling rate. 0.7% dextran containing bread was the softest with 39 and 27% lower crumb hardness on day 1 and day 4, respectively, compared to bread with SB only (Fig. 3). Staling rate, in turn, was the lowest (43 g/day) for the bread

containing the highest level (1.0%) of dextran, which also increased crumb resilience by 5 and 11% on day 1 and day 4, respectively. Lower dextran addition levels improved crumb resilience slightly but not significantly. The DSC results showed that dextran increased the onset temperature (T_o) on day 1 (from 43 °C to above 44 °C) and reduced the endset temperature (T_e) on day 4 (from 72 °C to 70–71 °C). The peak temperature (T_p) was not influenced by dextran. Compared to bread containing SB alone, dextran additions lowered the amylopectin melting enthalpy by 11–20% on day 1, however, the difference was statistically significant only with the highest addition level of dextran. On day 4, only the bread containing 0.7% dextran showed lower enthalpy change, although, not significantly different to bread with SB only.

The addition of MIMOs to the bread containing SB increased the dough yield and the bread yield by up to 3.4% and 3.6%, respectively, but did not change the weight loss during baking and cooling. The incorporation of MIMOs gradually decreased the SV of the bread, in relation to addition level. The lowest MIMO level (0.8%, MIMO 1) decreased the SV only by 2.4%, but the highest level (2.0%, MIMO 3) decreased it by 7.1%. MIMOs did not modify crumb hardness or resilience at any addition level but increased the staling rate from 51 to 55–58 g/day. The amylopectin melting behavior, in turn, was modified by the MIMOs. On day 1, the DSC onset temperature (T_o) was raised from 43 to 45–45.5 °C by MIMO addition, and the enthalpy change was 18–23% lower compared to bread containing SB only. The DSC measurement on day 4 showed that the impact of MIMOs was diminished and the amylopectin melting behavior was identical to bread containing SB only.

3.4. Correlation of the dough properties and the quality of bread

The relationship between the dough tests and bread quality parameters were analyzed with Pearson's correlation test. Correlations with 0.05 and 0.01 significance level were found and they are presented in Table 5. Higher water content in the dough was inversely correlated with L, BBS, R, hardness, and staling rate. SV of the bread was the best predicted (0.01 level) by P (inversely) and E but correlated (0.05 level) with the dough level and SHI as well. In addition to the dough water content, R and E/R ratio (inversely) were also correlated to bread crumb hardness and staling rate.

4. Discussion

In this study, dextran and MIMO were produced separately in water solution by dextransucrase preparation obtained from *W. confusa* A16 culture. The benefits of producing dextran and MIMO enzymatically instead of by fermentation involve a) avoiding acidification and other LAB metabolites, b) standardized dextran/MIMO synthesis, not dependent on fermentation kinetics, but where conditions can be optimized for the enzyme directly, and c) no "nutritional" requirements for incubation matrix composition, except for the substrate and $CaCl_2$. A traditional fermentation approach, in turn, has its benefits too, such as improved hygienic safety. Considering the SB material, pH drop by LAB fermentation can be useful to prevent spore-activation during the recycling process (Immonen et al., 2020; Weegels, 2010).

4.1. Dextran and MIMO production with dextransucrase preparation

The crude dextransucrase preparation had close to 8 U/ml enzyme activity. The enzyme activity, however, is strongly related to the composition of reaction mixture (Kim et al., 2003). Based on the measured enzyme activity, the incubation of 10% sucrose solution with 10% (v/v) enzyme preparation could theoretically consume all the sucrose in approximately 6h time, if the activity (fructose release) remained constant during the incubation. This was not the case in the incubation to produce dextran, where 2.7% sucrose remained in the solution even after 48 h of incubation. This is due to several factors, but mainly: 1) changing substrate composition along with the incubation and 2) production of dextran, which leads to increased viscosity. The viscosity caused by dextran formation reduces Brownian motion of particles in the solution. Subsequently, the enzyme-substrate collisions are reduced gradually due to both the increasing viscosity and reduced substrate. When maltose was added to the incubation mixture for MIMO production, no sucrose was left after 48 h incubation. The presence of maltose slightly (12%) increased the initial enzyme activity. More importantly, however, formation of MIMOs instead of dextran did not induce similar viscosity increase, thus, better maintaining the dextransucrase activity along with the incubation, which led to more efficient sucrose consumption and fructose release.

Panose yield in the MIMO solution was low (1.1 mg/g) and larger M_w MIMO represented most end products (Fig. 1). The sucrose-maltose ratio is often the defining factor behind the size distribution of MIMOs (maltose level up → M_w of MIMO down (Paul, Oriol, Auriol, & Monsan, 1986)), however, also the type and activity of dextransucrase, and conditions such as temperature and pH can influence the production of MIMOs. In varying maltose concentrations, panose yield was at its maximum when the maltose level was 110 mM and sucrose level 28 mM (Heincke, Demuth, Jördening, & Buchholz, 1999). The amount and activity of dextransucrase in relation to sucrose concentration, in turn, is inversely proportional to the M_w of produced dextran (Robyt, Yoon, & Mukerjea, 2008).

Table 5
Pearsons's correlation analysis table including main dough testing and bread quality parameters. Values are shown when the correlation is significant at 0.05 level, and bolded when significant at 0.01 level. Opt. WA = optimal water absorption, P = pressure, L = drum distance, BBS = bubble burst strain, SHI = strain hardening index, R = resistance to extension, E = extensibility, DL = dough level, SV = specific volume, H = hardness, SR = staling rate.

	Farinograph	Bi-axial extensional test					Uni-axial extensional test			Maturograph	Volume	Texture profile analysis		
Parameter	Opt. WA	P	L	P/L ratio	BBS	SHI	R	E	E/R ratio	DL	SV	H Day 1	H Day 4	SR
Opt. WA	1	–	**−0.852**	–	**−0.876**	–	**−0.915**	–	0.799	–	–	−0.738	−0.828	**−0.875**
P		1	–	**0.898**	–	−0.727	–	–	–	−0.719	**−0.851**	–	–	–
L			1	**−0.928**	**0.996**	–	–	–	–	0.740	–	–	–	–
P/L ratio				1	**−0.918**	−0.786	–	–	–	−0.745	–	–	–	–
BBS					1	–	–	–	–	0.708	–	–	–	–
SHI						1	–	0.744	–	0.753	0.747	–	–	–
R							1	–	**−0.906**	–	–	0.795	**0.856**	**0.853**
E								1	–	**0.875**	**0.843**	–	–	–
E/R ratio									1	–	–	**−0.960**	**−0.956**	−0.823
DL										1	0.786	–	–	–
SV											1	–	–	–
H Day 1												1	**0.966**	0.778
H Day 4													1	**0.914**
SR														1

The theoretical sum of carbohydrates in the dextran solution should be 100 mg/g (79.3 mg/g measured) and 125 mg/g in the MIMO solution (109.7 mg/g measured). Some systematic errors (due to sampling, standard- and analytical error) led to moderate underestimation of carbohydrate concentrations (Table 2). In addition to systematic error, leucrose formation is possible when free fructose concentration increases in the solution, which might partly explain the lower total-sugar content (Dols-Lafargue, Willemot, Monsan, & Remaud-Simeon, 2001; Paul et al., 1986). Furthermore, the MIMO concentration is calculated as panose equivalents, which might underestimate the MIMO concentration, because the specific detector responses of all MIMOs are not known. Despite the error sources mentioned, the results well describe the trend of carbohydrate redistribution in the solutions after incubation with dextransucrase. Although the linearity and M_w of the produced dextran was not determined in this study, we estimate that it is similar to that was previously determined for *W. confusa* A16 dextran (linear with only 3% of α-(1 → 3) linkages, and molar mass of 3.3×10^6 g/mol) obtained during growth on MRS agar plates (Wang et al., 2019). It has been shown that dextran produced by fermentation or by isolated enzyme of the same *Weissella confusa* E−90392 strain exhibit similar molecular features (Kajala et al., 2015).

4.2. Dough rheology and texture of model bread compared to wheat bread

The use of gluten-starch mixture as a simplified dough matrix allowed standard gluten content despite changing dough formulations. Hydration, properties of starch and gluten, and their proportions are known to affect gluten-starch dough rheology (McCann et al., 2018; Mohamed & Rayas-Duarte, 2003). The optimal consistency for the gluten-starch dough (14:86) was defined to be 360 FU after 5 min mixing in the Farinograph, instead of standardized 500 FU for wheat dough. Lower optimal consistency and WA are related to the lack of sugars, non-gluten proteins, and water-soluble polysaccharides such as arabinoxylans, which resulted in decreased viscosity of the gluten-starch aqueous phase compared to wheat dough. Moreover, accelerated starch retrogradation and water redistribution in the gluten-starch bread are attributed to lack of surface-active compounds, lipids, arabinoxylans, and the low initial moisture content. This was shown as higher hardness (208 g on day 1, 390 g on day 4) and staling rate (61 g/day) compared to the results obtained for wheat bread in our previous studies (hardness of 120–130 g on day 1 and 240–250 g on day 4, staling rate of 37–43 g/day (Immonen et al., 2020; Immonen et al., 2021).

4.3. The impact of surplus bread on dough rheology and bread texture

10% addition of SB increased the dough WA and changed the visco-elastic properties and proofing behavior of dough despite optimized dough hydration. The performed uni-axial and bi-axial extensional tests differ from each other's for three main reasons. 1) The direction of the extensional measurement, 2) different relaxation time prior to the measurement, and 3) stretching the dough to form either a string (uni-axial) or a film (bi-axial). These differences must be considered when interpreting the obtained results. In case of adding SB to the dough, although the bread was homogenized with water, bread particles (especially crust pieces) produce weak points to the dough film when inflated, thus potentially causing a premature bubble failure in bi-axial extension. This can be observed as significantly reduced drum distance values for all doughs containing SB. The increase in peak pressure (tenacity) and reduced extensibility due to SB addition indicates that the components in the SB form interactions with gluten-starch matrix and increase the viscosity of the aqueous phase in the dough. The strain hardening index and the E/R ratio, in turn, remained at the same level with control model dough. Therefore, the interactions between SB (especially gelatinized starch) and the gluten network can be presumed to hinder the optimal elasticity and gas-holding capacity of the gluten-starch dough. Moreover, as the strain hardening behavior of the dough was not modified by SB, it is likely that the entanglements between gelatinized starch and gluten-starch matrix compensate for the impaired gluten network. These entanglements, however, only occur in the early phase of dough deformation and seem to become irrelevant when the deformation proceeds, due to poor extensibility of the dough. The strain hardening behavior has been shown to well describe the gluten network functionality in wheat baking (Dobraszczyk et al., 2003). However, it is not known if the bread making performance of the dough can be predicted by strain hardening behavior that is increased by other compounds than gluten, such as hydrocolloids or starch. The interactions of SB components with gluten network led to impaired tolerance to mechanical stress (lower dough level) in the Maturograph test, and finally, to reduced SV of the bread. In our previous study, 10% addition of SB reduced wheat bread SV by 16%, increased day 1 hardness by 29%, day 4 hardness by 34%, and staling rate by 39% (Immonen et al., 2021). In this study, the reduction of SV by SB was 12.5%, and crumb hardness was not significantly changed. The staling rate was even lowered compared to CMB. Undoubtedly, the standardized gluten content improved the SV in relation to obtained results with wheat bread, but only to a limited extent. The different impact of SB on hardness and staling in gluten-starch bread can be attributed to overall faster crumb firming compared to wheat bread, as discussed above. Additionally, higher moisture content in the bread containing SB can maintain elasticity of the crumb structure longer which leads to retarded firming.

Differential scanning calorimetry was used to measure amylopectin crystal melting enthalpy which describes the rate of starch retrogradation during bread storage (Defloor & Delcour, 1999). Because of fast crumb firming in the gluten-starch bread, and faster retrogradation, most of the starch was already retrograded on day 4. Consequently, no clear difference in enthalpy change was obtained due to addition of SB alone.

4.4. The impact of dextran and MIMO on dough rheology and bread texture

Different dextran and MIMO addition levels (0.4–1.0% and 0.8–2.0%/FW, respectively) to the dough showed varying impact on dough and bread properties. In our previous study (Immonen et al., 2020), the amount of fermentation-produced dextran in the dough was approximately 1.3%/FW, which is slightly higher than the highest addition level in this study. MIMOs were also formed in the fermented waste bread; however, the amount was not quantified. In this study, dextran addition increased the optimal WA of the dough due to its hydrocolloid nature, which has already been reported by other researchers (Lacaze et al., 2007). Increasing WA led to a higher dough yield and partially influenced the changes in dough rheological properties and bread texture. Increasing moisture content is known to proportionally decrease the visco-elasticity of wheat dough (Masi, Cavella, & Sepe, 1998). Furthermore, strong gluten-hydrocolloid interactions have been proposed to limit the dough extension, as in the case of xanthan (Zannini et al., 2014).

Dextran modified the rheology of gluten-starch matrix containing SB by softening the dough, which was seen as reduced R and increased E/R ratio, meanwhile improving uni-axial extensibility. Softening of the dough by concurrently increasing dextran and water content also explains the decreasing trend in dough level during proofing under mechanical stress. Dextran at 0.7% level (DEX 2) showed higher extensibility and strain hardening index than other dextran addition levels, thus, indicating an optimal concentration for dough rheology. Consequently, 0.7% addition level produced breads with the highest SV and lowest hardness. The volume-improving and crumb softening trend could not be directly predicted by bi-axial extensibility, peak pressure, or P/L ratio that were not significantly influenced by dextran. Uni-axial extensibility and E/R ratio, in turn, better predicted the obtained bread volume and hardness, respectively (Table 5). This might be an outcome of longer dough relaxation and lesser impact of SB particles to the uni-

axial measurement. In the optimally hydrated dough, dextran and the water bound to it appeared to shield the gluten network from interactions by SB components, therefore, returning the SV of the bread to the CMB level. However, some beneficial entanglements between dextran and gluten network can be noticed, especially at 0.7% level, because the extensibility and strain hardening index were maintained at the CMD level despite substantially increased dough moisture content (dilution). It is also possible that the influence of dextran on dough gas cell stability becomes relevant during the early phase of baking due to changing solubility and water-binding properties of dextran, thus, leading to better oven rise and higher SV. Nonetheless, the interaction of dextran with gluten network appears to be aligning in nature rather than entangling, because of the obtained improvement in uni-axial extensibility but not in bi-axial extensional parameters. The volume-increasing effect of linear high-M_w dextran in wheat bread containing non-wheat flour ingredients was shown in this study accordingly with several reported studies (Immonen et al., 2020; Kajala et al., 2015; Wang et al., 2019).

The shielding effect may also explain the higher T_o and lower retrogradation enthalpy on day 1 DSC measurements. Starch retrogradation results on day 1 and 4 were not fully in line with crumb hardness results. The crumb-softening effect of dextran, therefore, cannot be explained by retarded starch retrogradation alone. Other textural features such as differences in structured network of macromolecules within the crumb and improved SV contribute to the crumb softness as well. Additionally, dextran bound higher amount of water to the dough and may have altered the redistribution of water during storage. This led to better crumb elasticity despite some of the water being bound to amylopectin crystallites. Earlier study showed that *in situ* produced dextran of *W. confusa* A16 can retard starch retrogradation in wheat-millet sourdough bread, but not compared to control wheat bread (Wang et al., 2019). Furthermore, pure high-M_w dextran alone or in combination with sourdough acidification, did not significantly reduce wheat bread retrogradation enthalpy after 7 days of storage, despite the reduction of crumb hardness (Zhang, Guo, Li, Jin, & Xu, 2019). Zhang et al. (2018) reported that high-M_w dextrans retarded bread staling rate up to 26%. In this study, the staling rate was reduced by almost 30% with 1.0%/FW (DEX 3) and by 25% with 0.7%/FW dextran addition.

The interactions with gluten proteins induced by MIMOs appeared to be stronger than those induced by dextran, as indicated by increased P/L ratio (Rosell, Rojas, & Benedito de Barber, 2001). The impact was particularly strong at low addition level of MIMOs, indicating that the interactions occur in relation to dough WA and MIMO concentration. MIMOs hindered the gluten network functionality by influencing the peak pressure and decreasing the strain hardening index and dough level. This hindering effect can be caused by direct interactions of MIMOs with gluten proteins, or by reinforcing the interactions of SB constituents and gluten-starch matrix. However, the reduction of starch retrogradation on day 1 and increased onset temperature (T_o) demonstrate that MIMOs can modify the amylopectin crystallization behavior. The influence of MIMO on retrogradation may have changed if a different MIMO size distribution was obtained. The lowered retrogradation did not result in lower crumb firmness. This may be due to other structural changes such as increased MIMO and SB interactions with gluten network throughout the bread crumb, and denser crumb structure due to lowered SV. The obtained results indicate that MIMOs produced by dextransucrase have a negative overall influence on baking performance of the gluten-starch dough containing SB. According to literature, however, MIMOs have shown plausible applications as dough improvers in frozen dough baking or in other non-baking related applications (Park, Jang, & Lim, 2016; Sorndech et al., 2018).

5. Conclusions

The addition of SB at 10%/FW level induced strong interactions between constituents of SB (especially gelatinized starch) and gluten network. This deteriorated the structure-forming of dough/bread which was observed as decreased extensibility, dough level, and SV, even though the gluten content was standardized, and WA optimized. Dextran addition at appropriate level (0.7%/flour weight) and optimized WA, shielded the dough gluten network functionality from the interactions of surplus bread. Uni-axial and bi-axial extensional results indicated that the specific interactions of dextran and gluten proteins are aligning in nature rather than entangling. MIMOs, especially at low concentration, induced stronger interactions than dextran with dough gluten proteins, which reinforced the negative impact of SB on the SV of bread. MIMOs did not reduce the overall crumb hardness despite partially preventing starch retrogradation in the early phase of storage. Among used dough tests, the uni-axial extensional test was the best suited to predict the baking performance of doughs containing SB and dextran or MIMO. The specific interactions of dextran and MIMOs with dough macromolecules are important to understand to develop suitable bioprocessing practices for bread making. The incorporation of enzymatically synthesized dextran to the bread dough can mitigate the negative influence of SB without concurrent acidification associated with *in situ* dextran synthesis by fermentation. Thus, in this study we established a practical method to enable SB recycling without compromising the technological quality of new bread. The dextran synthesis should aim at minimal formation of MIMO to achieve an optimally beneficial influence on volume and texture of the bread. However, the influence of MIMO should be further confirmed by using a wheat flour-based dough system.

Author statement

Mikko Immonen: Investigation, Methodology, Formal analysis, Writing-original draft, Funding acquisition. **Yaqin Wang:** Investigation, Methodology. **Rossana Coda:** Conceptualization, Writing-Review & Editing, Supervision, Funding acquisition. **Kati Katina:** Conceptualization, Methodology, Writing-Review & Editing, Supervision, Funding Acquisition. **Ndegwa H. Maina:** Conceptualization, Methodology, Writing-Review & Editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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