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Title

Waste bread recycling as a baking ingredient by tailored lactic acid fermentation

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Abbreviations

LAB, lactic acid bacteria; EPS, exopolysaccharide; SV, specific volume; CH, crumb hardness; CWB, control wheat bread;
IMO, isomalt-oligosaccharides; DW, dry weight; FW, fresh weight; f.w., flour weight
Abstract

Food-grade waste and side streams should be strictly kept in food use in order to achieve sustainable food systems. At present, the baking industry creates food-grade waste as excess and deformed products that are mainly utilized for non-food uses, such as bioethanol production. The purpose of this study was therefore to explore the potential of waste wheat bread recycling for fresh wheat bread production. Waste bread recycling was assessed without further processing or after tailored fermentation with lactic acid bacteria producing either dextran or β-glucan exopolysaccharides. When non-treated waste bread slurry was added to new bread dough, bread quality (specific volume and softness) decreased with increasing content of waste bread addition. In situ EPS-production (dextran and microbial β-glucan) significantly increased waste bread slurry viscosity and yielded residual fructose or glucose that could effectively replace the sugar added for yeast leavening. Furthermore, fermentation acidified waste bread matrix, thus improving the hygienic safety of the process. Bread containing dextran synthesized in situ by Weissella confusa A16 showed good technological quality. The produced dextran compensated the adverse effect of recycled bread on new bread quality attributes by 12% increase in bread specific volume and 37% decrease in crumb hardness. In this study, a positive technological outcome of the bread containing microbial β-glucan was not detected. The waste bread fermented by W. confusa A16 containing dextran appears to enable safe bread recycling with low acidity and minimal quality loss.

Keywords

Waste management; Cereal side stream; Bread quality; Exopolysaccharide; Dextran; β-glucan

Declarations of interest: none
1. Introduction

Food waste is a huge problem from a global food security and sustainability perspective (Gustavsson et al., 2011, Otles et al., 2015). In this paper, the term ‘waste’ includes all food-processing side streams that are currently disposed from manufacturing plants for non-food use. The total production volume of cereals produced in Europe is about 400 million tonnes/year off which roughly 5% is lost during processing (Gustavsson et al., 2011). In the Finnish baking industry, it is estimated that 5–10% of production volume (10–20 million kg annually) is wasted before entering retail. Considering current global undernourishment of over 800 million people, resource efficient practices should be implemented to avoid wasting edible food materials (FAO et al., 2014). Consequently, innovative ways to exploit side streams are crucial not only for cost efficiency but also for achieving sustainable food systems in a circular economy.

Bakery waste consists of both, food-grade materials that are mainly excess or defective ready products and materials that are unsafe for human consumption, such as dough waste or contaminated products. In the baking industry, several alternatives to reutilize waste have been developed. Bakery waste has been described to be suitable substrate for conversion into hydrogen (Adessi et al., 2018), bioethanol (Haroon et al., 2016), and biogas (Dubrovskis and Plume, 2017), or directly used as feed for livestock (Al-Ruqaie et al., 2011, Martínez San Pedro et al., 2015). In addition, several downstream processes have been suggested for waste bread in order to derive added-value chemicals for industrial use (Haroon et al., 2016). Future solutions must however focus on alternatives that retain or recycle food-grade bakery waste for human food use.

Commercial solutions available for bread re-utilization as food ingredient include bread usage as a brewing ingredient and bread recycling through untargeted fermentation. However, there is currently no scientific literature to describe in detail the process and impact of such recycling methods. Bread fermentation into new sourdough has been previously presented by Gélinas et al. (1999) and Weegels (2010). In-house recycling of industrially formed excess bread as a new baking ingredient is an appealing option to prevent a
substantial side stream being wasted and would be favored by EU and USA official waste management hierarchies (Otles et al., 2015). At present, there is no information available on how recycled bread affects the quality of new bread. There are two principal challenges in bread recycling as a dough ingredient. 1) Gelatinized starch and denatured protein presumably reduce the leavening potential of the dough posing a technological challenge. 2) Heat resistant Bacillus spp. spores, such as toxin-producing B. cereus and rope-forming B. subtilis and B. licheniformis, may be activated during recycling and induce a hygienic concern (De Bellis et al., 2015).

Lactic acid fermentation is a safe and practical way to acidify food materials and, therefore, prevent Bacillus spp. risk, since spores activation is prevented at low pH (below pH 5), (Valerio et al., 2012). Several LAB strains also produce exopolysaccharides (EPS) that increase the viscosity of the surrounding matrix. Some EPS have already been found to enhance the textural properties of breads (Ruas-Madiedo et al., 2002, Tieking et al., 2003) and hence, can be beneficial for enhancing baking properties of recycled waste bread. Particularly in situ produced dextrans have been shown to positively affect bread qualities such as increased loaf volume and retarded staling (Galle et al., 2012, Katina et al., 2009). Dextrans are α-glucan homopolysaccharides with varying molecular weight and degree of branching, commonly produced by LAB strains such as Leuconostoc spp. (Xu et al., 2017), Weissella confusa and Weissella cibaria (Wolter et al., 2014a). Generally, homo-EPS (including dextran) are synthesized by extracellular sucrase enzymes using sucrose as a substrate to produce EPS (α-glucan or fructan) (Kralj et al., 2002). Additionally, if suitable acceptor molecules such as maltose are present, isomalto-oligosaccharides (IMO) are formed (Leemhuis et al., 2013). β-glucan type homo-EPS, in turn, are synthesized by a transmembrane glucosyltransferase enzyme (similarly to hetero-EPS), which utilizes intracellular UDP-glucose as substrate (Werning et al., 2006). LAB such as Pediococcus species are known β-glucan EPS producers (Juvonen et al., 2015). Bacterial β-glucan EPS have been characterized as 2-substituted 1,3-β-D-glucan which make it structurally different from cereal or fungal β-glucans (Ibarburu et al., 2007, Juvonen et al., 2015). Neither dextran nor β-glucan EPS has been previously tested in bread recycling.
Characteristics of bread as a fermentation material are somewhat different from flour, but strongly dependent on formulation of the bread. Flour, which is the general raw material for sourdough, contains native protein and starch granules, active enzymes and various naturally occurring microorganisms. Bread, in turn, has gone through the baking process whereupon the starch is gelatinized (more accessible for microbial enzymes), enzymes inactivated, gluten proteins denatured and various new compounds formed due to yeast metabolism and heat. Micronutrient composition of wheat flour and wheat bread are similar together. Since bread is initially free of active microbes, fermentation of bread with a starter involves competition only against microbes originated from the environment after baking. In flour-based sourdoughs, pH often drops to around 4.0 or above, but not lower even though acidity continues to increase (Clarke et al., 2003). Weegels (2010), in turn, reported quick pH drop with low acidity while fermenting wheat bread. This indicates that pH buffering capacity of bread is lower compared to flour. Type of bread and water-bread ratio also affect to fermentation efficacy (Gélinas et al., 1999).

The purpose of the present study was to explore the potential of waste wheat bread recycling back to fresh wheat bread production. The aim was to introduce controlled fermentation as a tool to transform waste bread into EPS-enriched (dextran or β-glucan) bread slurry that can be utilized as a bakery ingredient. EPS-enrichment resulted in a significant amount of residual fructose or glucose in the slurry which were consequently used to replace sucrose that is added for yeast leavening in bread dough formulation. The fermentation was assessed by microbial growth, acidification and EPS-production (viscosity increase). Furthermore, the effect of the fermented waste bread slurry ingredient on bread quality parameters was evaluated in comparison to non-treated waste bread slurry.

2. Materials and methods

2.1. Raw material – waste bread

White wheat bread (French Country Bread, made with sourdough), was kindly provided by Koivulan Leipomo Oy, Kouvolan, Finland. The bread was composed of 45.6g starch, 9.3g protein, 3.2g fiber and 1.7g
fat/100g and kept at -20°C before use. The bread was grounded into <1mm crumbs, mixed with RO-water (1:3 w/w) and homogenized with a blender (Oster, Mexico) to form a “bread slurry”.

2.2. Lactic acid bacteria strains

Based on previous screening trials regarding EPS-producing efficacy (data not shown), two EPS-producing LAB strains, *Pediococcus clausenii* E-032355T (*P. clausenii* 55T), belonging to VTT Culture Collection (Teknologian Tutkimuskeskus VTT Oy, Finland) and *Weissella confusa* A16 (Wang et al., 2019) belonging to the Department of Food and Nutrition (University of Helsinki), respectively, were used. *W. confusa* A16 was used for dextran production while *P. clausenii* 55T was for β-glucan production. Additionally, *P. clausenii* E-022179 (*P. clausenii* 179) also belonging to VTT Culture Collection, was used as a non-EPS-producing *P. clausenii* strain possessing acidification rate similar to *P. clausenii* 55T. LAB strains were routinely propagated in MRS broth (LabM, Heywood, Lancashire, United Kingdom) at 30°C for 24h.

2.3. Fermentation trials

2.3.1. Fermentation conditions

Before inoculation, corresponding to 2% of the slurry volume, the bacterial cells were harvested from 24h MRS broth culture, by centrifuging at 10,000rpm for 10min. The cells were then suspended in phosphate buffer saline (PBS) (0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride, pH 7.4, Sigma-Aldrich, Saint Louis, USA) and centrifuged again. The PBS supernatant was discarded, the cells re-suspended in 4ml of RO-water and transferred into the bread slurry (initial LAB cell density of approximately 10^7 CFU g^-1 of slurry). When the bread slurries were further used for baking trials, the strains were grown in general edible medium (GEM, composition described by Wang et al. (2018)) at 30°C for 24h. The amount and condition of inoculum were the same as described above. All fermentations were carried out static at 25°C for 24h in two biological replicates. In order to optimize EPS-producing LAB growth in the slurry, the pH of the slurries were adjusted to pH 6 with 5M NaOH in the beginning of the fermentation. Along with controlled fermentations, a non-inoculated bread slurry was used as a control matrix and incubated in the same conditions. Waste bread slurries were prepared according to ingredient composition
Table 1. For EPS production, 10% (per slurry weight) sucrose was added to slurries fermented with W. confusa A16. To produce EPS-negative controls, bread slurries containing 5% glucose and 5% fructose instead of sucrose were also fermented with W. confusa A16. Glucose and fructose were added to maintain the sugar balance of the slurry and mimic the effect of LAB metabolism on slurry characteristics without EPS formation. For fermentations with P. claussenii 55T and 179, 6% glucose was added to the slurries for EPS production and maintaining the sugar balance after fermentation. Based on pre-trials (data not shown), 2% yeast extract (LabM) addition was required to enhance β-glucan production by P. claussenii 55T of at least 3-fold. On the contrary, yeast extract did not enhance W. confusa A16 EPS production and thus was not added to those fermentations.

2.3.2. Acidification and microbial growth

The pH and total titratable acidity (TTA) were measured from bread slurries at 0h and 24h. The pH-meter (Model HI 99161, Hanna Instruments, Woonsocket, RI, USA) and TTA titrator (EasyPlus Titration, Mettler Toledo, Columbus, OH, USA) were used for measurements as described by Wang et al. (2018). LAB growth was followed by CFU-counts at the beginning (0h) and end (24h) of the fermentation. In detail, bread slurry serial dilutions were made in 0.9% NaCl solution tubes. The diluted samples were then plated in MRS agar (LabM) and incubated at 30°C for 48h in microaerophilic conditions.

2.3.3. Organic acid analysis

Organic acids were analyzed from water/salt-soluble extract prepared from bread hydrolysates according to Weiss et al. (1993). After centrifugation (11 000rpm, 20min), 1ml of clear supernatant was collected and 1ml of 5% perchloric acid was added and vortexed thoroughly. The mixture was kept in 4°C overnight and then centrifuged (11 000rpm, 15min). 1ml of clear supernatant was then syringe-filtered (Acrodisc GHP Minispoke 45μm, Pall Corporation, USA) into HPLC vials. The analysis was performed on a Hi-Plex H column (Agilent Technologies, Inc., CA, USA; 300 × 6.5 mm), with a Hi-Plex H guard column (Agilent; 50 × 7.7 mm). The HPLC system was equipped with a Waters 515 pump (Waters Corp., Milford, MA, USA), autosampler, ultraviolet (UV) detector (Waters 717), and refractive index detector (HP 1047A, HP, USA) using 10 mM
H$_2$SO$_4$ as an isocratic mobile phase (0.5ml/min, run time 35min with 40°C column temperature). UV-chromatogram was used to quantify organic acids.

2.3.4. Dextran, oligosaccharide and free sugar analysis

Dextran content was measured from the slurries fermented by W. confusa A16. Dextran analysis was done with enzyme-assisted method according to Katina et al. (2009) and free sugars were measured from freeze-dried samples by HPAEC-PAD system. The HPAEC-PAD system consisted of autosampler (Waters 717 plus), three HPLC pumps (Waters 515), Dionex CarboPac PA1 Guard Column and Analytical Column (Thermo Scientific, Waltham, MA, USA) and electrochemical detector (Waters 2465). The free sugar and oligosaccharide profile measurements were performed for all samples with HPAEC-PAD according to Xu et al. (2017).

2.3.5. Viscosity measurement

The viscosity of bread slurries (indicating EPS production) was measured immediately after fermentations with RheolabQC Rheometer (Anton Paar, Austria) using a 27mm diameter probe (CC27) and at a constant temperature of 22°C. Flow curves were obtained from shear rate 3–100 S$^{-1}$ and back to 3 S$^{-1}$, and with 10 replicate measuring points in shear rate 100 S$^{-1}$. Viscosity measurements were performed in three analytical replicates, and the average viscosity at s100 S$^{-1}$ used for comparison on the samples.

2.4. Bread preparation

The composition of bread doughs is shown in Table 2. Non-treated bread slurries were added to doughs in three different levels 4.5%, 8.5% and 12.5% waste bread per flour weight (f.w.). Fermented bread slurries were added to the dough only at the highest (12.5%) addition level. Since the fermented slurries contained residual fructose and/or glucose, sucrose was not added to the bread doughs that contained added fermented slurry. Consequently, the residual monosaccharides from fermentation were used to compensate for the sucrose added in baking. To keep the dough yield and dry matter-water ratio the same in all breads, flour amount in the dough was reduced according to added waste bread amount (dry matter).
Control wheat bread (CWB) dough was prepared in each baking session and, therefore, results were derived as an average from nine CWB doughs. All other dough types were prepared as two replicate bakings.

Bread ingredients, flour, water, bread slurry, sugar, salt, yeast and fat were mixed 3 minutes slow and 4 minutes fast with a spiral mixer (Diosna Dierks & Söhne GmbH, Germany), followed by a floor time of 15 min (35°C, 75% relative humidity). The dough was then divided into 250g loaves and molded, followed by 45 min proofing (35°C, 75% relative humidity). The breads were baked in 200°C oven for 15 min with 15 seconds of steaming in the beginning. After one hour of cooling, the loaves were weighed, packed into plastic bags and stored in room temperature. Samples of bread were also stored in -20°C for pH and TTA measurements.

2.5. Bread quality measurements

Specific volume (SV) of the breads was measured from three breads during the day after baking using BreadVolSCAN laser scanner (Backaldrin, Austria). Texture profile analysis was performed for all breads with texture analyzer (TA-XT2i, Stable Micro Systems Ltd., UK) according to Wang et al. (2019). Crumb hardness (g) and springiness (no units, measured as relative difference in height of crumb cube between 1st and 2nd compression) of the breads were measured after storage at room temperature for 1 and 4 days. The pH and TTA were measured from the crumb of breads (stored in -20°C) as described by Wang et al. (2018).

2.6. Statistical analysis

Statistical analysis was performed with SPSS Statistics 25 software (IBM Corp., NY, USA) by one-way analysis of variance (ANOVA) and Tukey's post hoc test using p-value < 0.05.

3. Results and discussion

3.1. Fermentation trials

3.1.1. Acidification and microbial growth
The acidification and LAB growth results of the fermented slurries are shown in Table 3. The initial pH of the waste bread slurry was 5.2 since the bread originated from a sourdough process and thus contained 0.6 mg/g lactic acid. The initial LAB cell number in the non-treated bread slurry was 1.4 Log CFU g\(^{-1}\) that increased to 7.7 Log CFU g\(^{-1}\) after 24 h showing spontaneous LAB growth. However, the spontaneous LAB growth did not acidify or increase the viscosity of the slurry. The pH of the bread slurry was adjusted to 6.0 before inoculating the starter LAB to favour their growth. All the LAB strains multiplied roughly 1.5 log cycles, reaching a final cell density up to approximately 9 Log CFU g\(^{-1}\), after 24 h of fermentation and acidified the slurry to pH 4.0. Fermentation with W. confusa A16 yielded titratable acidity (TTA, ml of 0.1 M NaOH/10g sample) of 3.9 (EPS-pos.) and 3.4 ml NaOH (EPS-neg.), and 2.7–2.8 mg/g lactic acid concentration. P. claussenii strains, however, produced higher acidification compared to W. confusa A16, with the TTA of 10.2 ml NaOH and 7.8 mg/g lactic acid (P. claussenii 55T EPS-pos.), and TTA of 8.8 ml NaOH and 6.4 mg/g lactic acid (P. claussenii 179). The more intense acidification caused by fermentation with P. claussenii 55T and 179 strains might be due to the addition of yeast extract additionally providing free nitrogen to support microbial metabolism. In the pre-trials (data not shown) fermentation with P. claussenii 55T and 179 strains generated TTA of 5.0–6.0 ml NaOH and 2.5–3.5 mg/g lactic acid when yeast extract was not added. Added yeast extract also increased the buffering capacity by providing amphoteric compounds such as free amino acids and peptides to the system, which might explain why the pH of all fermented slurries was the same despite differing acidification rates between LAB strains. Unlike in flour fermentation, Bread slurry matrix itself is very inefficient in buffering the pH change compared to flour-water mixture, which enables a quick pH drop despite relatively low TTA content. Acetic acid concentration was not detected in all fermentations as expected (Galle et al., 2010). It is known that bread ingredients often contain contaminant microbes that can survive baking, such as Bacillus spp. spores, thus posing a hygienic concern (De Bellis et al., 2015). After the baking, Bacillus spp. spores can potentially activate if favorable conditions, such as high water activity, temperature (above 25°C) and pH (above 5) occur (Valerio et al., 2012). Possibly these conditions are prevalent when using the untreated bread slurry, making it an unappealing recycling option with respect to product safety. However, in pre-trials (data not shown) active Bacillus cereus was not detected in waste
bread slurries. The pH of the fermented bread slurries, in turn, dropped to pH 4, which effectively prevents *Bacillus* spore activation and therefore highlights the importance of fermentation during recycling.

3.1.2. Carbohydrate composition and viscosity

The waste bread did not contain significant amounts of mono- and disaccharides, only a residual of fructose and maltose, 0.8% and 3.0% DW, respectively (Table 3). Presumably the residual fructose originates from sucrose added to the original bread dough (waste bread), and maltose from the wheat flour used in breadmaking. Maltose concentration increased to 5.9% DW when the control slurry was incubated for 24h at 25°C, but fructose concentration remained unchanged. In EPS-positive slurry fermented by *W. confusa* A16, glucose was not detected, but 20% DW fructose was left as a residual from hydrolyzed sucrose. In EPS-negative slurry, about 18% DW of both glucose and fructose remained after fermentation. This means that less than 10% of both the added glucose and fructose, had been consumed during fermentation. Therefore, when prepared for the baking trials the glucose addition amount to bread slurry (5% per slurry fresh weight, FW) was reduced to 1% FW to maintain the sugar balance in baking.

Maltose concentration in *W. confusa* A16 EPS-positive and EPS-negative slurry was 0.4% and 1.4% DW, respectively, both being significantly lower compared to *P. claussenii* and control slurries. *W. confusa* A16 EPS-positive fermentation produced the most viscous slurry, 1.9 Pa*s, which was 7.6-times higher than its respective EPS-negative slurry viscosity. *W. confusa* A16 EPS-positive slurry viscosity was caused by *in situ* produced dextran that consisted 9.1% of slurry DW (equals to 2.33% FW dextran). Since the original amount of sucrose in the slurry was 10% FW and the dextran yield was 2.33% FW, thus, 46.6% of glucose from sucrose (sucrose-dextran conversion rate) was converted to dextran, resulting in 102g dextran/kg of waste bread. Dextran produced by *W. confusa* A16 strain has been previously shown to obtain high molecular weight and only few branches (< 3%) (Wang et al., 2019). The rest of the sucrose-derived glucose was bound to isomalto-oligosaccharides (IMO) and consumed by the LAB strain. Oligosaccharide formation was not detected in *W. confusa* A16 EPS-negative slurry, because sucrose was not present. In *W. confusa* A16 EPS-positive slurry, however, a clear series of IMO appeared in HPAEC-PAD chromatogram (Figure 1),
since both, sucrose and maltose were present. *W. confusa* A16 produces dextransucrase enzyme, which acts on sucrose yielding dextran, fructose and IMO. However, IMO formation generally requires an acceptor molecule like maltose (Leemhuis et al., 2013, Van Hijum et al., 2006). Dextranucrase adds released glucose units to maltose creating a series of different sized IMO (Hu et al., 2017), as can be seen also in the chromatogram of our *W. confusa* A16 fermented EPS-positive sample. This is also the reason why free maltose concentration was the lowest in *W. confusa* A16 EPS-positive sample. Furthermore, maltose concentration in the matrix is one key factor determining the sucrose-dextran conversion efficacy by channeling dextransucrase activity to produce IMO instead of dextran. Relatively low maltose concentration in waste bread partly enabled high sucrose-dextran conversion rate (46.6%). Obtained conversion rate was higher than previously reported in wheat flour-based sourdough (conversion rate of 32%)(Katina et al., 2009) and gluten free sourdoughs (conversion rate between 3.6% and 16.8%)(Wolter et al., 2014b). Millet sourdough, in turn, has been shown to have even 70% sucrose-dextran conversion rate (Wang et al., 2019).

For the slurries fermented by *P. claussenii* 55T and 179, approximately 22% DW glucose and 3% DW maltose remained in both slurries, which means that less than 10% of added glucose was consumed. Observed sugar consumption was similar to what was previously reported as sugar consumption per 24h while fermenting bread crumb, by Gélinas et al. (1999). *P. claussenii* 55T (EPS-pos.) slurry viscosity was 3.2-times higher than the respective *P. claussenii* 179 fermented slurry, due to β-glucan production (Juvonen et al., 2015). However, microbial β-glucan quantification in the complex bread matrix is currently not possible. Due to different biosynthetic mechanisms, dextran yield during fermentation is generally much higher compared to β-glucan yield (Dueñas et al., 2003, Hu and Gänzle, 2018). However, the glucose substrate for β-glucan synthesis could be derived directly from waste bread starch by enzymatic starch hydrolysis instead of added glucose. The enzymatic hydrolysis of bread starch has already been studied by Hudečková et al. (2017) and Sükrü Demirci et al. (2017).

3.2. *Baking trials*
The basic quality attributes of wheat bread are flavor, specific volume (SV) and textural properties. Consumers expect wheat bread loaves to be well leavened, airy, soft, and have a long shelf life; thus the basic requirement for bread recycling is that these quality attributes would remain unchanged. For typical yeast-leavened wheat breads, sucrose is usually added to the dough as a substrate for yeast metabolism to efficiently produce CO$_2$. Sucrose, or glucose, is also a substrate for LAB EPS synthesis, which is why in this study the LAB fermentation was tailored to end up with the suitable amount of sugar, residual fructose ($W$. confusa A16) or glucose ($P$. claussenii 55T and 179) left as a substrate for yeast leavening. Therefore, additional sucrose was not needed to the bread doughs containing fermented slurry. All baked breads maintained the shape of a regular wheat bread. However, changes were observed in the sizes of bread loaves, as confirmed by SV (Figure 2) and CH (Figure 3) measurements, as well as in crust color.

When control waste bread slurry was added to breads, both, SV and CH deteriorated with increasing content of slurry addition. The SV of breads with added bread slurry decreased consistently, 5%, 12% and 20% compared to control wheat bread (CWB), when more waste bread was added (Figure 2). The quality-impairing trend was also clear in terms of CH after 1 and 4 days of storage, as well as for the calculated staling rate (CH increase/day), which was 41g/day for CWB and 72g/day for the bread with 12.5% waste bread addition (Figure 3). The pH of CWB and 12.5% slurry addition breads were pH 5.6 and 5.4, respectively, and TTA was 2.9 and 3.2 ml NaOH, respectively. Springiness differences between all bread types were very minor, being 0.97–0.98 after 1 day of storage and 0.94–0.96 after 4 days of storage. Considering the substantial quality loss of the recycled breads, direct addition of untreated waste bread slurry was clearly not a favorable recycling method. During baking, the native starch of wheat flour is gelatinized, which significantly changes starch functional properties and might explain why direct recycling of waste bread yields impaired quality bread. In the study by Ortolan et al. (2015), pre-gelatinized starch addition to wheat bread was shown to reduce SV and increase CH, similarly to the present experiment. Interestingly though, pre-gelatinized starch (extruded flour) was considered to benefit frozen dough baking, by absorbing moisture (Ortolan et al., 2015), and gluten free baking by providing structure to maintain gas cells (Pedrosa Silva Clerici et al., 2009). In addition to gelatinized starch, bread contains denatured wheat
proteins that are not taking part again in gluten network formation of the new dough. Since a part of flour is replaced by added waste bread, there is slightly less native protein available for gluten formation, which might be another factor that yields to impaired quality attributes. Even though loss of SV and increase in CH should be avoided in wheat bread baking, slight changes in SV and CH may not be problematic for bread types such as rye bread or wheat-based flat bread.

The pH of breads containing slurry fermented by W. confusa A16 was 4.9–5.0 and TTA approximately 4.0, that is mildly acidic compared to typical sourdough wheat breads. The pH and acidity of all breads were in line with the acidity results measured in fermented slurries (Table 3). The SV of W. confusa A16 EPS-negative bread was only around 3.3ml/g (equal to unfermented slurry addition bread), but W. confusa A16 EPS-positive bread, which contained dextran, had a significantly higher SV of 3.7ml/g (Figure 2) compared to the other breads. Compared to untreated slurry addition bread (12.5%), W. confusa A16 EPS-positive bread had 12% higher SV, and 37% lower CH after both 1 and 4 days of storage. W. confusa A16 EPS-positive bread was also the only bread with day 1 and day 4 CH not significantly different to CWB, although it was slightly lower in springiness. Therefore, it seems that the produced dextran (2.3% in the slurry, 0.7% in bread dough), and potentially the IMO, can significantly improve recycled bread textural quality.

Compared to CWB, the in situ produced dextran can entirely compensate the negative effect of waste bread addition on CH and staling, and partially (about 50%) compensate the loss of SV. The positive effect of in situ produced dextran on bread quality is well-established (Katina et al., 2009, Lynch et al., 2018, Wolter et al., 2014a). Beneficial effects of dextran are likely due to water binding in breadcrumb and interaction with both, gluten network and gelatinized starch, thus assisting in bread structure formation and retarding starch retrogradation. The role of IMO on bread technological properties remains still unclear, however, IMO are known to act as prebiotics (Sorndech et al., 2018). The dextran amount produced during fermentation could be potentially increased, if maltose was removed, for instance, with enzymatic treatment. Without maltose present, larger portion of glucose derived from sucrose would end up in dextran (Lynch et al., 2018).
The breads containing slurry fermented by *P. claussenii* 55T and 179 had clearly darker crusts compared to other breads. This was presumably due to more intense Maillard-reaction caused by added yeast extract, containing free amino groups, and free glucose. Generally, quicker crust browning is considered beneficial for bakeries, because it enables lower oven baking temperature and, thus, energy saving. The pH of the above breads was 4.4–4.5 and TTA 5.8–6.1 ml NaOH. The SV and CH results of 55T and 179 breads were similar (Figures 2 and 3), meaning that the *in situ* produced β-glucan did not show any effect on these quality attributes. Compared to bread with added untreated slurry (12.5%), the SV of breads containing slurries fermented by *P. claussenii* 55T and 179 were no different but both breads were significantly (roughly 20%) softer, yet harder than bread with dextran of *W. confusa* A16 and CWB, being around 29% harder on day 1 and 38% harder on day 4. Improved softness is likely due to yeast extract addition to *P. claussenii* slurries, and other differences related to LAB metabolism occurring during fermentation, such as stronger acidification (Katina et al., 2006). Despite the 3.2-fold increase of slurry viscosity, the amount of β-glucan produced may not have been sufficient to affect bread properties. The amounts of produced bacterial β-glucan remain generally very small (100–200mg/l), as quantified previously in synthetic growth mediums (Garai-Ibabe et al., 2010, Dueñas et al., 2003).

4. Conclusions

Wheat bread recycling back to new bread production is challenging, and requires tailored processing in order to maintain good technological properties of the bread. The present study uncovers novel perspective about the potential of valorizing waste bread as an EPS-enriched baking ingredient. LAB fermentation is strongly suggested as a recycling tool due to the acidification of the bread matrix and thus potential improvement of its hygienic safety. Of the tested fermentation conditions, bread containing dextran synthesized *in situ* by *W. confusa* A16 showed good technological quality. *In situ* produced dextran compensated the adverse effect of recycled bread on new bread quality attributes. In this study we were not able to detect a positive technological outcome of the bread containing microbial β-glucan. Several other bioprocessing practices, such as tailored enzymatic treatments and targeted fermentations could be
studied for waste bread recycling creating novel and functional food ingredients. In order to implement the findings into practice, bakeries could optimize the recycling process to match suitable fermentation time, temperature and waste bread/water-ratio as well as test the stability of fermentation continued by backslopping. In addition, a traceability system should be applied to recycling for monitoring product constituents, allergens among others. The waste bread fermented by W. confusa A16 containing dextran enables safe bread recycling with low acidity and minimal quality loss, while containing prebiotic IMO, and residual fructose available for yeast leavening.

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6. Tables

*Table 1.* Ingredient composition of non-treated and fermented waste bread slurries

|                     | Control slurry | *Weissella confusa* A16 | *Pediococcus clausenii* |
|---------------------|---------------|-------------------------|-------------------------|
|                     | Weight (g)    | EPS-pos. Weight (g)     | EPS-neg. Weight (g)     | Strain 55T Weight (g) | Strain 179 Weight (g) |
| Waste bread         | 200           | 200                     | 200                     | 200                   | 200                   |
| Water               | 600           | 600                     | 600                     | 600                   | 600                   |
| Sucrose             | -             | 89                      | -                       | -                     | -                     |
| Glucose             | -             | -                       | 44.5                    | 53.4                  | 53.4                  |
| Fructose            | -             | -                       | 44.5                    | -                     | -                     |
| Yeast extract       | -             | -                       | -                       | 17.4                  | 17.4                  |
| Target LAB amount   | -             | 10⁷                     | 10⁷                     | 10⁷                   | 10⁷                   |
| Incubation time     | 24h           | 24h                     | 24h                     | 24h                   | 24h                   |
| Incubation temperature | 25°C   | 25°C                    | 25°C                    | 25°C                  | 25°C                  |
**Table 2.** Recipes for control wheat bread, breads with added non-fermented and fermented waste bread. Non-treated waste bread slurry was added by 4.5%, 8.5% and 12.5% level, describing the percent quantity of recycled waste bread per flour weight (f.w.). Fermented slurry addition amounts were adjusted to match 12.5% waste bread per f.w.

| Bread type | Flour Weight (g) | Water % f.w. | Bread Slurry Weight (g) | Salt 1.5% | Sucrose** 2.8% | Yeast 5.0% | Fat 6.0% | Total 174.7 |
|------------|-----------------|-------------|-------------------------|----------|----------------|-----------|---------|-------------|
| Control wheat bread                      | 1400          | 100.0       | 843                     | 21       | 28             | 70        | 84      | 2446        |
| Non-treated bread slurry additions 4.5% | 1353          | 96.7*       | 637                     | 252      | 21             | 39        | 84      | 2457        |
| Non-treated bread slurry additions 8.5% | 1312          | 93.7*       | 455                     | 476      | 21             | 39        | 84      | 2457        |
| Non-treated bread slurry additions 12.5%| 1264          | 90.3*       | 279                     | 700      | 21             | 39        | 84      | 2457        |
| Weissella confusa A16 EPS-pos. 12.5%    | 1264          | 90.3*       | 279                     | 778      | 21             | -         | 84      | 2496        |
| Weissella confusa A16 EPS-neg. 12.5%    | 1264          | 90.3*       | 279                     | 747      | 21             | -         | 84      | 2465        |
| Pediococcus claussenii E-032355T 12.5%  | 1264          | 90.3*       | 279                     | 760      | 21             | -         | 84      | 2478        |
| Pediococcus claussenii E-022179 12.5%   | 1264          | 90.3*       | 279                     | 760      | 21             | -         | 84      | 2478        |

*The amounts of flour being replaced by waste bread addition were 3.3%, 6.3% and 9.7% respectively.

**Sucrose was not added to the doughs containing fermented slurry because residual fructose or glucose in the fermented slurries was sufficient for yeast leavening.
| Variable    | Unit       | Time | Non-treated bread slurry | Weissella confusa A16 | Pedicoccus clausenii |
|------------|------------|------|--------------------------|-----------------------|----------------------|
|            |            |      | 0h                       | EPS-pos.              | EPS-neg.             | Strain 55T | Strain 179 |
| pH         |            | 0h   | 5.2                      | 6.1*                  | 6.0*                 | 5.9*       | 5.9*       |
|            |            | 24h  | 5.1                      | 4.0                   | 4.0                  | 3.9        | 4.0        |
| TTA        | ml NaOH/10g| 24h  | 1.9 ± 0.0                | 3.9 ± 0.1             | 3.4 ± 0.1            | 10.2 ± 0.1 | 8.8 ± 0.1  |
| Lactic acid| mg/g       | 24h  | 0.7 ± 0.0                | 2.8 ± 0.1             | 2.7 ± 0.0            | 7.8 ± 0.1  | 6.4 ± 0.1  |
| LAB count  | Log CFU g⁻¹| 0h   | 1.4 ± 0.12               | 7.3 ± 0.02            | 7.3 ± 0.03           | 7.4 ± 0.03 | 7.4 ± 0.03 |
|            |            | 24h  | 7.7 ± 0.03               | 8.9 ± 0.06            | 9.0 ± 0.04           | 8.8 ± 0.11 | 9.0 ± 0.11 |
| Viscosity  | Pa*s       | 24h  | 0.22 ± 0.01ᵇ             | 1.90 ± 0.06ᵇ          | 0.25 ± 0.05ᶜ         | 0.41 ± 0.02ᵈ | 0.13 ± 0.01ᵃ |
| Glucose    | % per dry weight | 24h | 0.8 ± 0.1ᵃ | 20.1 ± 0.7ᵇ | 18.5 ± 0.3ᵇ | 22.4 ± 0.5ᵇ | 22.1 ± 0.4ᵇ |
| Fructose   | % per dry weight | 24h | 5.9 ± 0.1ᵈ | 0.4 ± 0.1ᵃ | 1.4 ± 0.3ᵇ | 3.4 ± 0.2ᶜ | 3.3 ± 0.3ᶜ |
| Maltose    |            | NA   | 9.1 ± 0.2ᵃ | 0.2 ± 0.0ᵇ | NA          | NA          |            |
| Dextran    |            | NA   | NA                      | NA                    | NA                    | NA          |            |

ND = not detected, NA = not analyzed

*pH adjusted to approximately 6.

**Viscosity as measured at shear rate of 100 [1/s].

Different letter after each number indicate significant difference (P<0.05 one way ANOVA) between values within each row.
7. Figure captions

Figure 1. HPAEC-PAD chromatograms (oligosaccharide analysis) of waste bread slurry water-soluble extracts: Non-treated bread slurry 0h (A), Non-treated bread slurry 24h (B), Pediococcus clausenii 55T fermented slurry (C), P. clausenii 179 fermented slurry (D), Weissella confusa A16 EPS-positive fermented slurry (E) and W. confusa A16 EPS-negative fermented slurry (F). Oligosaccharide formation occurred only during W. confusa A16 fermentation with sucrose present (EPS-positive fermentation) as can be seen by the peaks in the oligosaccharide region (retention time between 25 and 45 minutes).

Figure 2. Specific volumes of control wheat bread, breads with non-treated bread slurry addition (4.5%, 8.5%, 12.5%) and breads with fermented slurry addition (12.5%). W.= W. confusa A16 fermented slurry bread, P.= P. clausenii fermented slurry bread. Different letters on the top of the columns indicate significant difference (P<0.05 one-way ANOVA) between bread types.

Figure 3. Crumb hardness of control wheat bread, breads with non-treated bread slurry addition (4.5%, 8.5%, 12.5%) and breads with fermented slurry addition (12.5%) after 1 day and 4 days of storage. W. = W. confusa A16 fermented slurry bread, P. = P. clausenii fermented slurry bread. Different letters on the top of the columns indicate significant difference (P<0.05 one-way ANOVA) between bread types.
8. Figures

![Diagram of chromatograms showing separation of maltose and oligosaccharides across six panels labeled A to F.](image-url)
Highlights

- Direct recycling of bread had a negative effect on bread quality
- Waste bread was successfully fermented with exopolysaccharide-producing LAB
- Dextran-enrichment improved waste bread reusability
- Microbial β-glucan-enrichment had no effect on bread quality