International Journal of Food Microbiology 302 (2019) 69–79

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Improvement of taste and shelf life of yeasted low-salt bread containing functional sourdoughs using *Lactobacillus amylovorus* DSM 19280 and *Weisella cibaria* MG1

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ARTICLE INFO

Keywords:
Antifungal
Exopolysaccharide
Low-salt bread
Salt reduction
Functional sourdough
Descriptive sensory

ABSTRACT

The challenge remains for the baking industry to reduce salt levels in yeasted bread as directed by governments, retailers and consumers around the world. The two main problems associated with the reduction of salt are a lack of salty taste and the reduction in shelf-life. Both of these issues are addressed in the presented work. A range of breads containing different levels of salt (0.0%, 0.3% and 1.2% of NaCl) in combination with various levels of sourdough (0%, 6%, 12%, 18%, 24%) was produced. The different doughs were analysed for their rheological behaviour. The bread quality characteristics such as loaf volume, crumb structure, staling rate and microbial shelf life were also determined. The sourdoughs were analysed for their different metabolites: organic acids, sugars, exopolysaccharides (EPS), and antifungal compounds. A trained sensory panel was used to perform descriptive analysis of the bread samples. The object of this paper is to use functional sourdoughs, containing *Lactobacillus amylovorus* DSM 19280 and *Weisella cibaria* MG1 to compensate for the quality problems that occur when salt is reduced in yeasted bread. The application of functional sourdoughs containing exopolysaccharides and/or antifungal substances in salt reduced breads significantly improved the quality. The application of functional sourdoughs allows the reduction of salt to a level of 0.3%.

1. Introduction

The excessive intake of sodium by consumers in developed countries is linked to several health related issues. In particular, cardiovascular diseases were identified as the most concerning impact on human health (Brown et al., 2009; Farquhar et al., 2015; O'Donnell et al., 2015). Hence, a reduction of salt in the western diet has been targeted in all industrialised countries. High sodium intake is a leading cause of cardiovascular diseases and hypertension and has also been linked to an increased risk of stroke, stomach cancer, kidney disease and bone demineralization (Wardener and MacGregor, 2002). The effect of sodium on hypertension is dose dependent; the more sodium consumed, the greater the increase in blood pressure (Panel on Dietary Reference Intakes for Electrolytes and Water, 2005). From a public health perspective, this makes controlling hypertension related to sodium intake an important issue. Global organisations (WHO and FAO, 2003) as well as national authorities (European Commission, 2008; FSAI, 2005; German Nutrition Society (DGE), 2009; Korean Food and Drug Administration, 2007; SACN, 2003) have put salt reduction on their agenda. The intake of sodium increased mainly due to the increase in the processed food being consumed (Gibson et al., 2000; Reddy and Marth, 1991; SACN, 2003). Bread as a staple food, is one of the main food products targeted by health organisations and can contribute up to 40% of the dietary sodium depending on the country and the respective culinary tradition (Angus, 2007; Cauvain and Young, 2007; Thomson, 2009).

The health related necessity of salt reduction in bread leads to a number of challenges for bakers and the bread industry. A range of bread characteristics are affected by the salt reduction, such as taste profile (Lynch et al., 2009), crumb structure (Beck et al., 2012a) and

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https://doi.org/10.1016/j.ijfoodmicro.2018.07.015
Received 23 April 2018; Received in revised form 15 June 2018; Accepted 11 July 2018
Available online 12 July 2018
0168-1605/

shelf-life (Filtenborg et al., 1996; Pateras, 2007; Samapundo et al., 2010). Breads with reduced amounts of NaCl were described as more sour/acidic and yeasty (Lynch et al., 2009). Beck et al. (2012b) reported significant changes in crumb structure based on different amounts of NaCl. As previously reported (Belz et al., 2012a, 2012b), NaCl reduction from 1.2% to 0.3% NaCl reduces the shelf-life of bread by approximately two days.

Numerous different strategies have been proposed to reduce the sodium chloride content of foods. Among them (i) taste enhancers (such as amino acids, monosodium glutamate, lactates, yeast products, soy based ingredients), (ii) salt replacer (KCl), (iii) the enhancement of sodium chloride perception by taste contrast (spatially separated different sodium chloride concentrations or the use of coarse-grained sodium chloride), and (iv) sourdough application (thanks to the combined effect of LAB acidification and proteolysis occurring during the fermentation) are those mainly investigated (Silow et al., 2016). Technologically, a reduction in salt to 0.6–0.3% would be feasible without a significant deterioration in rheological properties or performance during manufacture (Lynch et al., 2009). However, its effect on the organoleptic properties of bread is still a critical factor in consumer acceptance.

The present paper focuses on the determination of the minimum amount of sourdough needed to compensate for the reduction of NaCl in yeasted wheat bread. Based on previous research two functional lactic acid bacteria strains were considered; the antifungal strain *Lactobacillus amylovorus* DSM19280 (Axel et al., 2015; M.C.E. Belz et al., 2012a, 2012b; Ryan et al., 2011) as well as the exopolysaccharide producer *Weisella cibaria* MG1 (Galle et al., 2010, 2011; Wolter et al., 2014). The impact of the sourdough on the dough and bread quality was analysed. Rheological analyses were performed on the dough samples based on sourdough addition. The changes of the different bread characteristics were also evaluated to optimise the quality of low-salt bread.

2. Materials and methods

2.1. Bacteria and growth conditions

The exopolysaccharide (EPS) producing strain *W. cibaria* MG1 and the antifungal strain *L. amylovorus* DSM 19280 were previously isolated from gluten-free sourdough (Arendt et al., 2009; Moroni et al., 2011a, 2011b). The bacteria were grown on MRS5 (Meroth et al., 2003) agar plates for 48 h at 30 °C and a single colony was transferred into MRS5 broth for about 16 h at 30 °C.

2.2. Sourdough fermentation

For sourdough fermentation, a pre-culture of the respective strains was subcultured in 40 mL of MRS5 broth and incubated for 24 h at 30 °C; *W. cibaria* MG1 and *L. amylovorus* (DSM19280) resulted in a cell suspension containing approximately 5×10^9 CFU/ml. Cells were harvested by centrifugation at 3000*g* for 10 min, washed twice with Ringer's solution and re-suspended in 40 mL of the same solution. Sourdough was prepared with a dough yield of 200 and an inoculation of about 10^7 CFU/g of sourdough using 600 g of wheat flour, 560 mL of sterile distilled water and 40 mL of cellular suspension. After mixing with a Kenwood mixer (Kenwood KM020) using the batter attachment for 1 min. at speed 1, the dough was covered and fermented at 30 °C for 24 h for the *W. cibaria* MG1 sourdough and at 30 °C for 48 h for the *L. amylovorus* DSM19280 sourdough. At the end of fermentation, lactic acid bacteria cell counts were evaluated on MRS5 agar plates and the total titratable acid (TTA) and pH values were determined according to Arbeitsgemeinschaft Getreideforschung e.V. (AFG, 1994). For the *W. cibaria* MG1 sourdough samples TTA = 7.6 ± 0.2 mL, pH = 4.26 ± 0.04 and for the *L. amylovorus* DSM19280 sourdough samples TTA = 17.8 ± 0.9 mL, pH = 3.75 ± 0.04 were measured. Both strains showed cell counts of about $5 \times 10^8 \pm 10^3$ CFU/g. These parameters were used as the "stability" parameter of sourdough fermentation.

2.3. Baking procedure and loaf analysis

Wheat bread was prepared by mixing Baker's flour (Odlums, Ireland), distilled water (water level set to 64.7% (flour weight) using a Brabender farinograph), dry yeast (Puratos Group, Belgium) and NaCl at levels of 1.04, and 0.26% (w/w) with a spiral mixer (Kenwood KM020). Considering an average bake loss of 13.5%, the NaCl concentrations in the final bread loaves resulted in 1.2 and 0.3%. Sourdough levels of 6, 12, 18, 24% were added to the different samples for sourdough fermented with both *L. amylovorus* DSM19280 and *W. cibaria* MG1. After a bulk fermentation of 15 min. at 30 °C and 85% relative humidity (Koma Popular, Koma, Roermond, The Netherlands) 450 ± 1 g bread loaves were moulded with a moulding machine (Machinefabriek Holtkamp B.V., Almelo, Holland) and put into pans. The loaves were proofed for 75 min under the same conditions used during bulk fermentation. Subsequently, the breads were baked for 35 min at 230 °C top and bottom heat in a deck oven (MIWE condo, Arnstein, Germany). Ovens were pre-steamed (0.3 L) and steamed when loaded (0.7 L). Loaves were depanned and cooled for 120 min at room temperature. The breads were baked with the different addition levels of sourdough and NaCl as shown in Table 1. The specific loaf volume was measured using a Volscan Profiler (Stable Micro Systems, UK). A TA-XT2i texture analyser (Stable Micro Systems, Surrey, UK) equipped with a 25 kg loading cell and a cylindrical aluminium probe (diameter of 35 mm) was used to analyse the texture profile of the bread crumbs. Crumb grain was described by the following parameters: slice brightness, number of cells, porosity expressed as the area of cells (the total area of cells as a percentage of the total slice area), wall thickness (the average thickness of cell walls) and the average cell volume, using a C-cell bread imaging system (Calibre Control International Ltd., UK).

Table 1
Overview of the prepared bread recipes.

	Control breads (no sourdough)						Sourdough breads (low-salt)											
	Standard salt			Low-salt			6% sourdough			12% sourdough			18% sourdough			24% sourdough		
	% FW[a]	% DW[b]	g	% FW[a]	% DW[b]	g	% FW[a]	% DW[b]	g	% FW[a]	% DW[b]	g	% FW[a]	% DW[b]	g	% FW[a]	% DW[b]	g
Baker's flour	100.0	59.5	1785	100.0	60.0	1799	95.0	57.0	1709	90.0	54.0	1619	85.0	51.0	1529	80.0	48.0	1439
Baker's yeast	2.0	1.2	36	2.0	1.2	36	2.0	1.2	36	2.0	1.2	36	2.0	1.2	36	2.0	1.2	36
Water	64.3	38.2	1147	64.3	38.5	1156	59.3	35.6	1067	54.3	32.5	976	49.3	29.6	887	44.3	26.6	797
NaCl	1.75	1.04	31.2	0.44	0.26	7.9	0.44	0.26	7.9	0.44	0.26	7.9	0.44	0.26	7.9	0.44	0.26	7.9
Sourdough	0.0	0.0	0	0.0	0.0	0	10.0	6.0	180	20.0	12.0	360	30.0	18.0	540	40.0	24.0	720
Total amount	168.0	100.0	3000	166.7	100.0	3000	166.8	100.0	3000	166.7	100.0	3000	166.7	100.0	3000	166.7	100.0	3000

[a] Flour weight.
[b] Dough weight.

2.4. Analyses of organic acids and sugars

An Agilent 1260 high performance liquid chromatography (HPLC) system equipped with a refractive index detector (RID) and an ultra violet-diode array detector (UV/DAD) was used to quantify carbohydrates (0.125–2.5 mM), organic acids (2–32 mM) as well as antifungal compounds (5–50 ppm). Standard calibration curves were prepared with 5 different concentrations and measured in duplicates always at the beginning and end of a sample set. Calibration curves showed good linearity with correlation coefficients of 0.999 for all compounds. For acid and sugar analyses freeze-dried sourdough samples were extracted with water and proteins were precipitated with Carrez solutions (White, 1979). After centrifugation (2000 ×*g*, 20 min) and filtration (0.2 μm), sugars were quantified over the RID (35 °C) by elution of the extract from a Hi-Plex H column (300 × 7.7 mm, 8 mm, Agilent, Cork, Ireland), equipped with a guard column (50 × 7.7 mm, 8 mm, Agilent, Cork, Ireland), using water at a flow rate of 0.6 mL/min at 25 °C. Setting the UV/DAD at 210 nm, lactic acid and acetic acid in the sourdough were determined after elution with 0.004 M sulphuric acid at 65 °C from the same column and a flow of 0.5 mL/min. Injection volumes were 20 μL.

2.5. Analyses of antifungal compounds

The sample preparation was performed as described by Brosnan et al. (2012). Sourdough samples were freeze-dried and ground to a fine powder. These freeze-dried sourdough samples (2.0 g ± 0.01 g) were weighed into individually labelled polypropylene tubes (50 mL) and H_2O (10 mL) was added and vortexed for 30 s. The samples were then fortified with deuterated internal standard (100 μL) and left to stand for 15 min. Ethyl acetate (EA) (10 mL) with 0.1% formic acid (FA) was dispensed into the samples which were then vortexed for 30 s. NaCl (1 g) and $MgSO_4$ (4 g) were added and shaken immediately upon addition for 1 min. The samples were then centrifuged for 10 min at 3500 rpm (2842 ×*g*). The organic supernatant containing the targeted compounds was transferred to a 15 mL Agilent dSPE tube, vortexed for 30 s and centrifuged for 10 min at 3500 rpm (2842 ×*g*). A 5 mL aliquot of the supernatant (equivalent to 1/2 of the original samples; 1.0 g) was transferred to a 15 mL polypropylene tube with 500 μL of dimethyl sulfoxide (DMSO) and evaporated under nitrogen at 50 °C on a Turbovap LV system. Extracts were filtered through 0.2 μm PTFE 13 mm millex syringe filters (Millipore) and 5 μL was injected onto the ultra-high pressure liquid chromatography (UHPLC)-MS/MS system.

Separations were performed using a Waters (Milford MA, USA) Acquity UPLC system employing an Acquity BEH shield RP18 analytical column (2.1 × 100 mm, particle size 1.8 μm) maintained at a temperature of 50 °C and the pump was operated at a flow rate of 0.6 mL/min. A binary gradient system was used to separate analytes comprising of mobile phase A, 0.1% acetic acid in water and mobile phase B, 0.1% acetic acid in acetonitrile. The gradient profile was as follows: (1) 0–2 min, 95% A, (2) 2–5 min, 70% A, (3) 5–7 min, 0% A, (4) 7–7.5 min, 0% A, (5) 7.51–11 min, 95% A. The UHPLC autosampler was sequentially rinsed using strong and weak washes that consisted of methanol/isopropanol/water (80/10/10), and water/methanol (80/20) respectively. These washes were required to clean the needle and reduce the carryover between injections. Antifungal compounds were detected using a Waters Quattro Premier triple quadrupole instrument operated in negative electrospray ionisation mode (Milford, MA, USA). The UHPLC-MS/MS system was controlled by MassLynx™ software and data were processed using TargetLynx™ software (both from Waters). The electrospray voltage was set at 2.5 kV in negative mode. The desolvation and source temperatures were set at 400 and 150 °C, respectively. Nitrogen was employed as the desolvation and cone gases and was set at 1000 and 50 L h^{-1}, respectively. Argon was employed as the collision gas at a flow rate of 0.21 μg mL^{-1} – which typically gave pressures of 3.52×10^{-3} mBar. The MS conditions were optimised by teed infusion of 10 μg mL^{-1} standard solutions into 50% mobile phase A and B at a flow rate of 20 μL min^{-1} and 0.2 mL/ min, respectively. A validation of the method was performed in compliance with the EC (EU, 2002) and ICH (ICH, 2005) guidelines taking into account specificity, linearity, limits of detection and quantitation, trueness and precision. The validation was completed by analysing standard concentrations (1 ppm, 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, 50 ppm) in triplicate on the first day and over three consecutive days. Controls (2.5 ppm, 12.5 ppm 17.5 ppm and 30 ppm) were run three times each on the first day and over three consecutive days. Signal to Noise (S/N) values of S/N = 3 was selected to determine the limit of detection (LOD) and S/N = 10 used to calculate the limit of quantitation (LOQ).

2.6. Shelf-life antifungal activity in bread

For the microbial shelf life tests the “natural” bakery-environment was used for contamination. Breads were sliced and 10 slices of each type of bread were exposed to the bakery-environment for 10 min. Each of the bread slices were packaged in polyethylene bags and heat sealed. The sterile air exchange was enabled by inserting two filter tips in each storage bag. During storage the temperature was kept constant at 20 °C. The storage took place up to 14 days observing the appearance of mould spoilage every day. As soon as spoilage appeared on one of the bread slices, the previous day was determined as the shelf-life of that bread (Belz et al., 2012a, 2012b).

2.7. EPS analysis

In order to determine the amount and molecular size of EPS, the isolation of water-soluble polysaccharides from flour and sourdough samples was carried out as described (Galle et al., 2010). Freeze dried samples were reconstituted in distilled water to a final concentration of 2 mg/mL. EPS were detected with a refractive index detector (RID) and their molecular weight was estimated using two dextrans; low molecular weight (LM) dextran with a relative molecular weight $M_r = 10^5$–2×10^5 and high molecular weight (HMW) dextran ($M_r = 5 \times 10^6$–4×10^7) and inulin from chicory ($M_r = 10^4$) for calibration (all obtained from Sigma Aldrich, Oakville, Canada). EPS size was determined by asymmetrical flow field-flow-fractionation (FFF) coupled to multi-angle light scattering (MALS) and a refractive index (RI) detector (Postnova, Salt Lake City, UT) as described by Galle et al. (2010 and 2011). The channel dimensions were 335 × 60 × 40 mm (Postnova) with a molecular weight cut-off of 10 kDa. A polyetherketone pre-column filter unit placed between channel and detectors contained a 2 μm pore-size and a Teflon microfilter (0.1 μm poresize) of regenerated cellulose filter paper (all Postnova). The samples were injected onto the channel at a flow rate of 0.2 ml/min and a cross flow of 2 ml/min for 1 min. After injection, the cross flow rate of 2 ml/min decreased exponentially to 0.1 ml/min over 30 min and was then maintained at 0.1 ml/min for 30 min. The molecular weight was measured by static light scattering data as processed by the AF 2000 software (Postnova, Salt Lake City, UT) with the RI signal as concentration detector. A dn/dc (specific refractive index increment) value of 0.147 was used for light scattering calculations of EPS. Polystyrolsulphonate standards (Postnova) and bovine serum albumin (Sigma Aldrich) were used for calibration of detectors. Sugars were analysed with a Carbopac PA20 column (Dionex, Oakville, Canada) using water (A), 200 mM NaOH (B) and 1 M Na-acetate (C) as solvents at a flow rate of 0.25 mL/min with the following gradient: 0 min. 30.4% B, 1.3% C, 22 min. 30.4% B, 11.34% C followed by washing and regeneration. Sucrose, glucose, fructose, maltose, panose, isomaltose, isomaltotriose (all obtained from Sigma Aldrich) and isomaltooligosaccharides (IMO, from Bionutra Inc., Edmonton, Canada) were used as external standards.

2.8. Fundamental rheology

Freshly prepared dough pieces (14 g) were sealed in airtight

Table 2
Sourdough metabolites sugars, lactate, acetate, TTA, pH and exopolysaccharides (EPS).

Sourdough metabolites [mmol kg^{-1}]/flour	Fructose	Glucose	Sucrose	Maltose	Lactate	Acetate	TTA [ml]	pH	EPS [g kg^{-1}]/sourdough
Wheat flour, unfermented	n/d	n/d	45.5 ± 0.4	48 ± 2	n/d	n/d	2.2 ± 0.0	6.1 ± 0.1	1.7 ± 0.4
Weisella cibaria (MG1)	23 ± 2	49 ± 4	n/d	198 ± 4	139 ± 4	73 ± 3	7.6 ± 0.2	4.26 ± 0.03	4.5 ± 0.3
L. amylovorus (FST 2.11) DSM19280	25 ± 2	13 ± 2	n/d	181 ± 5	360 ± 7	n/d	17.8 ± 0.9	3.75 ± 0.04	n/d

containers and allowed to rest for 10 min before loading. Measurements were conducted using a controlled stress/strain rotational rheometer (MCR301 Anton Paar GmbH, Germany). Parallel serrated plates with a diameter of 25 mm were used set at a temperature of 30 °C. The measurements were performed at a final gap of 1.000 mm after a relaxation rest of 10 min (Addo et al., 2001). Fundamental rheological properties were determined within the linear viscoelastic range (LVR). Frequency sweeps were conducted in the range of 1–100 Hz at a constant strain of 0.01%. The complex modulus (G*) data were obtained from the frequency sweeps and the weak gel model was applied using the equation $G^*(\omega) = A_F \cdot \omega^{1/z}$ according to (Gabriele et al., 2001). All results are averages of two measurements of three independent samples.

2.9. Sensory

Sensory analysis was carried out by a panel of eight assessors, recruited according to international standards (ISO, 2014). At the start of the study, the panel took part in a number of focus groups for the development and refinement of a lexicon to describe the sensory characteristics of the breads. During these focus groups, all bread samples included in the study were examined and a list of attributes describing their odour, appearance, texture and flavour characteristics was generated. These terms were refined in subsequent sessions and the most representative terms describing the samples were retained. Descriptive sensory analysis was carried out using a final vocabulary of 5 odour ("roasted", "sourdough", "cheesy", "doughy", "yeasty"), 1 appearance ("density"), 1 texture ("density") and 6 flavour ("yeasty", "doughy", "musty/stale", "roasted", "salt", "bitter") terms which showed a level of significance of $p < 0.05$.

The descriptive testing took place over a period of two days with a total of 11 breads analysed in duplicate. The order of tasting was balanced to account for the order of presentation and carry-over effects (MacFie et al., 1989). Ahead of the assessment, the assessors were provided with deionised water, a list of the defined vocabulary and instructed to cleanse their palate between tastings. Breads were scored for attributes on unstructured 100 mm line scales labelled at both ends with extremes of each attribute. The intensity of each of the descriptive terms was recorded for each sample using the Compusense ® five V. 4.0 sensory data acquisition programme (Guelph, Ontario, Canada). The descriptive analysis yielded duplicate data matrices consisting of 8 assessors by 13 sensory attributes by 11 breads. The mean panel scores from the duplicate descriptive sensory analysis were subjected to one-way analysis of variance (ANOVA, SPSS v 14.0 SPSS Inc. Chicago, Illinois, USA) to determine which terms were effective at providing discrimination among the breads at $p \leq 0.05$. All descriptive terms significantly discriminated ($p < 0.05$) between the samples and were included in subsequent analyses. Data were averaged across replicates, standardised (1/standard deviation) and analysed by means of principal component analysis (PCA) using Guideline +7.5 (CAMO ÅS, Trondheim, Norway). The way in which each principal component (PC) discriminated between all bread samples was determined by performing an ANOVA (SPSS v 14.0 SPSS Inc.) on the PCA scores prior to averaging across replicates. The final number of components for interpretation was based on the discriminating ability ($p \leq 0.05$) of each PC and a visual inspection of explained validation variance (to indicate whether additional PCs were modelling information or noise).

2.10. Statistics

Statistical analyses were performed using MiniTab 16 for Windows computerised statistical analysis package (MiniTab Ltd., Coventry, UK). Data were examined using the one-way analysis of variance (ANOVA). Where an F-test showed significant differences ($p < 0.05$), Fisher's least significant difference (LSD) test was used for multiple comparisons. Each result is the average of at least three separate experiments with three independent samples from each batch.

3. Results and discussion

3.1. Sourdough metabolites analyses

Sourdoughs were prepared, as previously described, by fermenting Baker's flour with two selected lactic acid bacteria strains *Weisella cibaria* MG1 and *Lactobacillus amylovorus* DSM19280. For both sourdoughs, started with the two LAB strains, the targeted metabolic traits, such as sugars, organic acids, total titratable acids (TTA) and pH, exopolysaccharides and antifungal compounds production were determined.

The unfermented wheat flour contained, 45.5 mmol sucrose and 48 mmol maltose per 1 kg baker's flour. Neither single sugars nor acids were detected. With a TTA of 2.2 ml and a pH of 6.1 the flour had a standard quality as described by Souci et al. (2000). The sourdough fermented with the heterofermentative *Weisella cibaria* MG1 strain contained fructose as well as glucose as intermediate metabolites. All the measured concentrations of metabolites (Table 2) are in line with the results published by Galle et al. (2010). The added sucrose was completely metabolised. The acidification level was low resulting in a TTA = 7.6 ml, pH = 4.6 in agreement with the concentrations of acetic acid (73 mmol kg^{-1} flour) and lactic acid (139 ± 4). The presence of maltose, initially in the flour and during the fermentation process released by the amylase activity, favoured the metabolism of sucrose and led to an increased EPS synthesis (Galle et al., 2010). Furthermore, the analyses on exopolysaccharides (EPS) resulted in 4.5 ± 0.3 g kg^{-1} sourdough as previously described by (Galle et al., 2010).

The fermentation with the *Lactobacillus amylovorus* (DSM19280) strain resulted in a highly acidified sourdough with a pH = 3.75 ± 0.04 and a TTA = 17.8 ± 0.9 (Ryan et al., 2011). Due to the homofermentative characteristics of *Lactobacillus amylovorus* strains lactic acid (360 ± 7 [mmol kg^{-1}]/flour) was the only acid which could be determined. All sucrose originating from the wheat flour was metabolised. The amount of maltose increased significantly due to the high amylase activity of the *L. amylovorus* (Axel et al., 2015). No EPS could be determined in the sourdough containing *L. amylovorus*.

3.2. Quantification of antifungal acid compounds

The selected *L. amylovorus* (DSM19280) had proven to compensate the reduced shelf life of bread due to reduced levels of salt (Belz et al., 2012a, 2012b). The antifungal acidic compounds were determined for both types of sourdough using HPLC-MS/MS analyses (Fig. 1) The determined amount of quantified compounds was significantly higher for the *L. amylovorus* DSM19280 strain than for the *W. cibaria* MG1 with the exception of vanillic, caffeic and hydro-p-coumaric acid (phloretic acid). This correlates with the determined antifungal impact of the *L.*

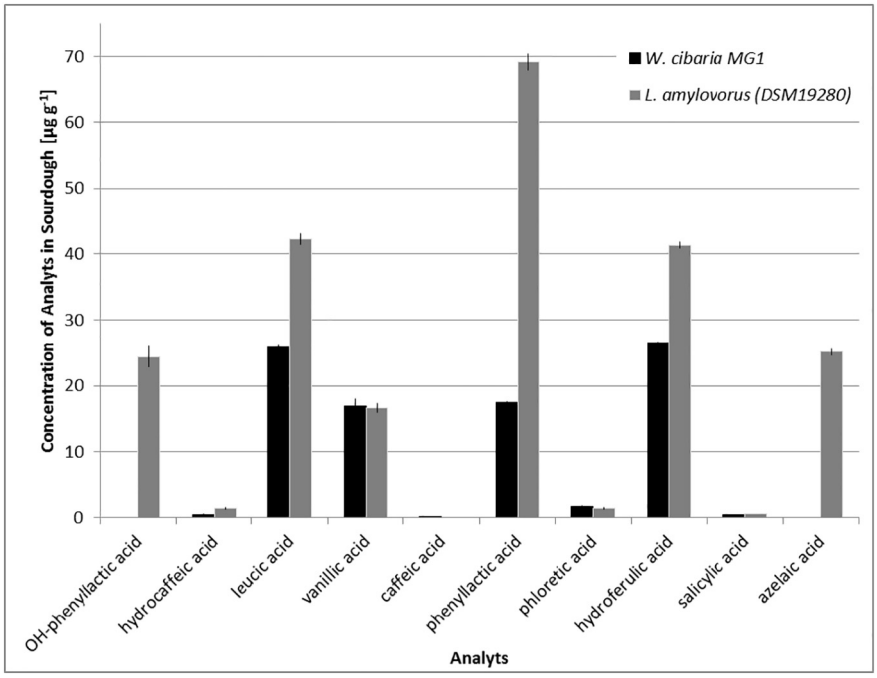

Fig. 1. Quantification of antifungal acid compounds in sourdough fermented using L. amylovorus (DSM19280) (■) and W. cibaria MG1 (■). The analyses were performed using a UPLC-MS/MS.

amylovorus strain on the bread samples prolonging their shelf life. As previously reported for different fermented substrates such as grass silage inoculated with *L. plantarum* (Broberg et al., 2007) or wort fermented with a strain of *L. brevis* (Axel et al., 2014), the concentrations of the various compounds were too low to be effective based on the known minimum inhibition concentrations (MIC) (Aziz et al., 1998; Ryan et al., 2011). However, the production of additional antifungal compounds, such as antifungal peptides (Axel et al., 2015) cannot be excluded. Complex synergistic effects can explain the determined antifungal activities in the various studies. The analyses resulted in the detection of 9 antifungal compounds within the *L. amylovorus* sourdough sample, while 15 different compounds have been identified previously in cell free supernatant of this strain (Ryan et al., 2011). The discrepancy between the two studies can be explained by taking into account that two different substrates (sourdough and cell free supernatant) were analysed. Vermeulen et al. (2006) showed that the production of antifungal compounds is substrate specific. While caffeic acid has been reported to show no antifungal activity as an isolated compound (Bisogno et al., 2007; Kim et al., 2004), all of the other determined compounds are known for their antifungal activity (Axel et al., 2015; Broberg et al., 2007; Guo et al., 2012). The compounds 3-phenyllactic acid and 4-hydroxyphenyllactic acid have previously been detected as antifungal substances produced by *L. plantarum* and several studies have proven the antifungal effect in sourdough and sourdough breads (Broberg et al., 2007; Dal Bello et al., 2007; Lavermicocca et al., 2003; Ryan et al., 2009; Ström et al., 2002). Leucic acid was reported to inhibit the growth of *Candida* and *Aspergillus* species in broth (Sakko et al., 2014) and also the azelaic acid had been isolated from *L. reuteri* as an antifungal compound by Guo et al. (2012).

3.3. Fundamental rheology

Rheological frequency sweeps were performed to determine the impact of the sourdough addition on the microstructural characteristics of the bread dough samples (Table 3). The weak gel model was applied to determine the resistance to deformation A_F and the network connectivity z of the dough samples over the frequency range of ω = 1–100 Hz. This model was introduced by Gabriele et al. (2001) and is based on the power equation $G^*(\omega) = A_F \bullet \omega^{\frac{1}{z}}$. The frequency sweeps of all dough samples showed a viscoelastic behaviour which means all measured elastic moduli (G′) were higher than the corresponding viscous moduli (G″) (Dobraszczyk and Morgenstern, 2003; Hoseney and Rogers, 1990; Song and Zheng, 2007). The reduction of NaCl from standard salt to low-salt led to a significant increase of dough strength of 29% as well as to a significant increase in network connectivity. On the contrary, Beck et al. (2012a) described a decrease of dough strength for reducing amounts of NaCl for large scale deformation using a farinograph. This was explained by the interaction of sodium and chloride ions with the positive and negative side chains of the gluten protein molecules favouring the network formation between the protein molecules. Our result can be explained by the known differential behaviour between small-scale and large-scale deformation rheological properties of wheat dough (Lynch et al., 2009; Mann et al., 2005; Tronsmo et al., 2003). In actual fact, there is still a gap of knowledge concerning how to relate small-scale deformation and large-scale deformation. For all low-salt samples fermented with *W. cibaria* the resistance to deformation decreased significantly with a linear correlation to the sourdough concentration. The fermentation with *L. amylovorus* resulted in a similar picture whereby the values were insignificantly lower. The stronger acidification of the *L. amylovorus* strain led to a more intense degradation of the gluten network resulting in a decreased dough strength. The network connectivity z did not differ between the two sourdoughs for lower addition levels. When sourdough was added at 24%, the network connectivity significantly differed, impacting on dough network. There are two main reasons for those changes of dough properties upon sourdough addition: firstly, being a positive net charge due to the presence of the organic acids which caused an unfolding of the gluten proteins (Galal et al., 1978) and secondly, an activation of the endogenous proteinase naturally present in wheat flour during sourdough fermentation (Bleukx et al., 1997; Thiele et al., 2002). Both effects led to a weaker integrity of the gluten network (Bleukx and Delcour, 2000). Comparing the samples containing the two different sourdoughs, it shows clearly that the acidification and the degradation of the flour polymers are the cause for the drop in dough strength and the EPS has no measurable impact on it. On the contrary, the presence of EPS caused a significant reduction of the network connectivity while

Table 3
Dough strength towards deformation (AF) and network connectivity (z) are shown for bread dough samples containing *W. cibaria* MG1 and *L. amylovorus* (DSM19280). The frequency sweep was performed at an angular frequency range of ω = 1–100 Hz and a constant target strain of γ = 0.01% (after the weak gel model of Gabriele et al. (2001)).

NaCl concentration	Dough samples	A_F	ΔA_F [%]	z	R^2
1.04%	Standard salt control	4122 ± 115^{a,c}		3.8 ± 0.1^{a}	0.996 ± 0.002
0.26%	Low-salt control	5298 ± 418^{b}	29%[1]	4.6 ± 0.1^{c}	0.99990 ± 0.00004
	6% *W. cibaria* MG1	4663 ± 231^{a}	−12%[2]	4.46 ± 0.08^{c}	0.9998 ± 0.0001
	12% *W. cibaria* MG1	3934 ± 150^{c,d}	−26%[2]	4.4 ± 0.4^{b,c}	0.996 ± 0.006
	18% *W. cibaria* MG1	3480 ± 260^{d}	−34%[2]	4.3 ± 0.2^{a,b,c}	0.9997 ± 0.0004
	24% *W. cibaria* MG1	2590 ± 304^{e}	−51%[2]	4.05 ± 0.05^{a,b}	0.99991 ± 0.00003
	6% *L. amylovorus* (DSM19280)	4354 ± 190^{a,c}	−18%[2]	4.45 ± 0.05^{b,c}	0.99993 ± 0.00002
	12% *L. amylovorus* (DSM19280)	3974 ± 272^{a,c,d}	−25%[2]	4.40 ± 0.08^{b,c}	0.99995 ± 0.00002
	18% *L. amylovorus* (DSM19280)	3437 ± 285^{d}	−35%[2]	4.42 ± 0.07^{b,c}	0.9992 ± 0.0007
	24% *L. amylovorus* (DSM19280)	2399 ± 382^{e}	−55%[2]	4.3 ± 0.1^{b,c}	0.9997 ± 0.0002
	6% *L. amylovorus* + 18% *W. cibaria*	3346 ± 311^{d}	−35%[2]	4.34 ± 0.06^{a,b,c}	0.9998 ± 0.0004

Values followed by a different letter are significantly different ($p < 0.05$).
[1] Relating to standard salt control.
[2] Relating to low-salt control.

Table 4
Specific bread loaf volume and bread crumb characteristics.

NaCl concentration	Dough samples	Specific bread volume	Number of cells	Porosity (area of cells)	Wall thickness	Cell volume
		[ml/g]		[%]	[mm]	[mm^3]
1.04%	Standard salt control	2.7 ± 0.2^{a}	3620 ± 148^{a}	53.8 ± 0.4^{a}	0.466 ± 0.006^{a,b}	8.2 ± 0.5^{a,b}
0.26%	Low-salt control	3.0 ± 0.3^{a,b}	3564 ± 103^{a}	54.8 ± 0.3^{b,c}	0.488 ± 0.005^{c,d}	9.5 ± 0.4^{c,d}
	6% *W. cibaria* MG1	3.1 ± 0.2^{b,d}	3827 ± 158^{a}	54.9 ± 0.5^{b,c,d}	0.477 ± 0.008^{b,c}	8.9 ± 0.4^{a,b,c}
	12% *W. cibaria* MG1	3.1 ± 0.2^{b,d}	3831 ± 99^{a}	54.9 ± 0.3^{c,d,e}	0.482 ± 0.006^{c,d,e}	9.1 ± 0.3^{c,d,e}
	18% *W. cibaria* MG1	3.4 ± 0.2^{c}	3703 ± 80^{a}	55.9 ± 0.4^{e,f}	0.499 ± 0.005^{d,e}	10.2 ± 0.5^{d,e}
	24% *W. cibaria* MG1	4.1 ± 0.1^{d}	3712 ± 90^{a}	56.8 ± 0.3^{f}	0.505 ± 0.004^{e}	10.7 ± 0.3^{e}
	6% *L. amylovorus* (DSM19280)	3.0 ± 0.3^{b}	3378 ± 118^{a}	55.0 ± 0.6^{a,b,c,d,e}	0.493 ± 0.007^{c,d,e}	10.2 ± 0.8^{b,c,d,e}
	12% *L. amylovorus* (DSM19280)	3.0 ± 0.2^{a,b}	3556 ± 76^{a}	55.5 ± 0.3^{c,d,e}	0.498 ± 0.007^{d,e}	10.4 ± 0.5^{d,e}
	18% *L. amylovorus* (DSM19280)	3.1 ± 0.3^{d}	3686 ± 102^{a}	55.9 ± 0.3^{d,e}	0.491 ± 0.008^{c,d,e}	10.0 ± 0.8^{c,d,e}
	24% *L. amylovorus* (DSM19280)	2.8 ± 0.0^{a}	4142 ± 194^{b}	54.1 ± 0.8^{a,b}	0.459 ± 0.011^{a}	7.9 ± 0.8^{a}
	6% *L. amylovorus* + 18% *W. cibaria*	3.4 ± 0.3^{c}	3642 ± 174^{a}	55.1 ± 0.3^{c,d,e,f}	0.497 ± 0.006^{d,e}	10.0 ± 0.3^{c,d,e}

Values followed by a different letter are significantly different ($p < 0.05$).

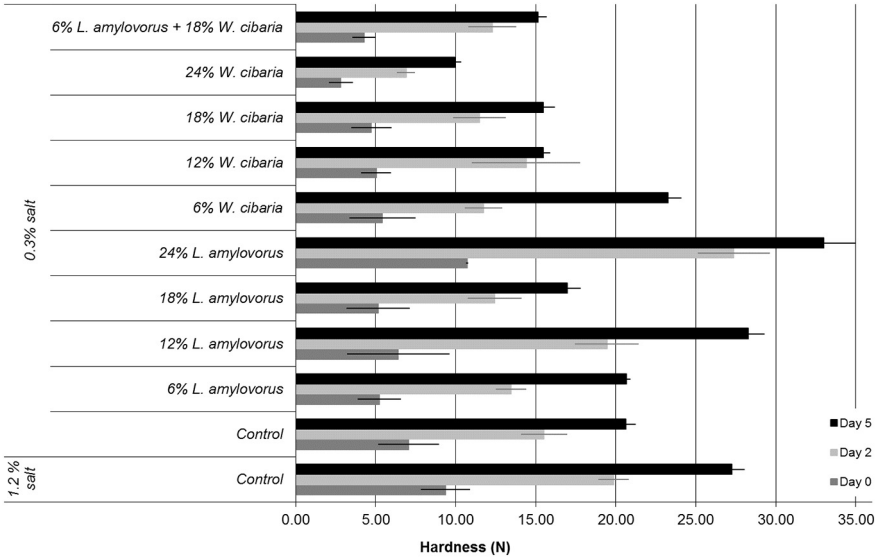

Fig. 2. Hardness of bread crumb on day 0, 2 and 5 after baking. Control breads were analysed as well as sourdough bread containing W. cibaria or L. amylovorus at concentrations of 6, 12, 18 and 24% and the combination of both sourdoughs at optimum levels.

the organic acids did not change the network integrity significantly. According to the weak gel model the EPS impacts on the network connectivity, reducing the interactions between starch and protein molecules (Gabriele et al., 2001; A. V. Moroni et al., 2011b).

3.4. Bread analyses

The baked bread loaves were analysed measuring the specific loaf volume as well as the specific characteristics of the crumb grain structure; namely slice brightness, number of cells, porosity, wall

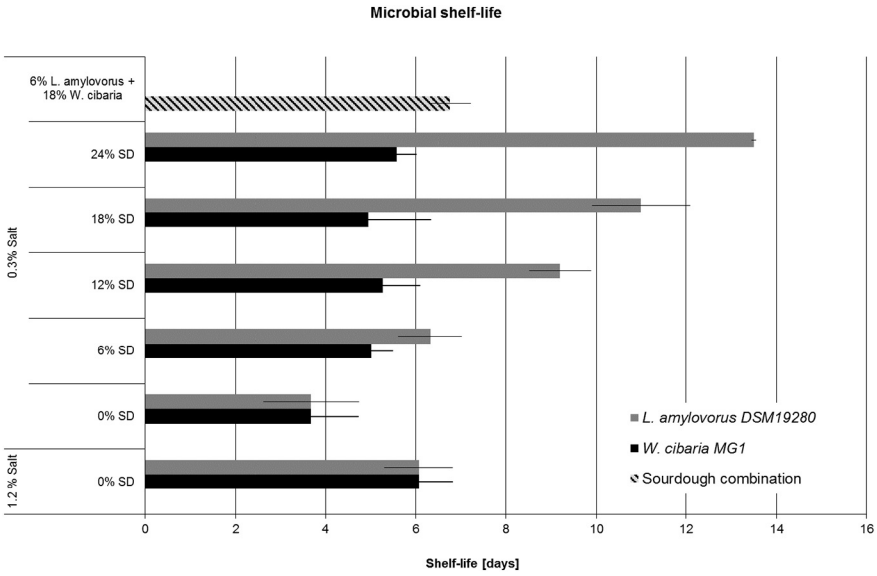

Fig. 3. Microbial shelf-life of bread slices exposed to the bakery environment. The following bread samples were considered: standard salt and low-salt breads without SD, sourdough breads at low salt level with sourdough addition of 6%, 12%, 18% and 24% for both sourdough; 6% L. amylovorus (DSM19280) and 18% W. cibaria MG1.

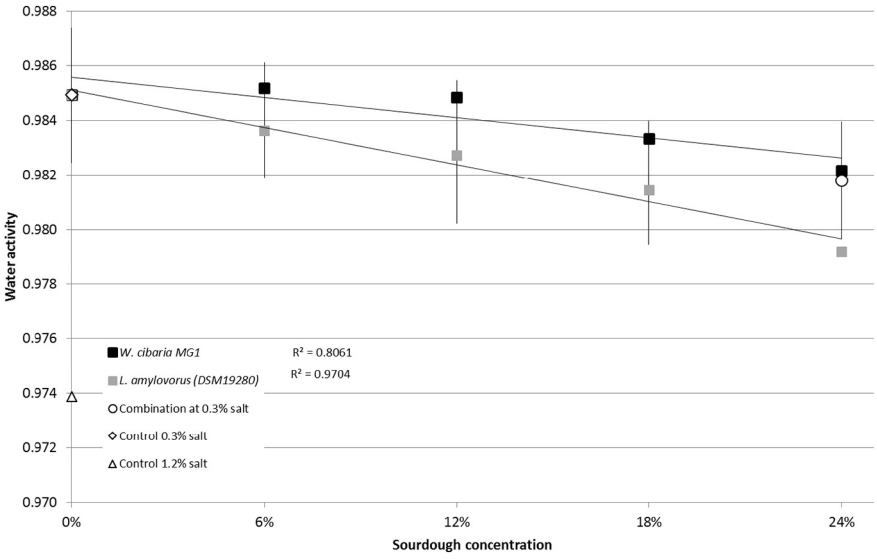

Fig. 4. Water activity of control breads at standard salt (Δ) and low-salt levels (◊), L. amylovorus breads (■) and W. cibaria breads (■) at low-salt level as well as the sourdough combination of 6% L. amylovorus DSM19280 with 18% W. cibaria MG1 (○).

thickness and cell volume. Salt reduction caused significant differences for crumb porosity, wall thickness and cell volume. This is in line with previous research published by Lynch et al. (2009) who could show a significant increase of porosity between 1.2% and 0.3% NaCl. The reduction of NaCl resulted to an increased yeast activity in bread dough during fermentation and hence, causing a more open and porous crumb structure (Cauvain, 2007). The increased wall thickness is based on a smaller number of larger cells surrounded by heavier cell walls (Czuchajowska et al., 1989).

The increasing addition of *W. cibaria* sourdough caused significant increases of loaf volume (Table 4). As little as the interaction of EPS, gluten and starch is understood, the positive impact of EPS on the specific bread volume has been proven in several research papers (Di Monaco et al., 2015; Galle et al., 2010; Katina et al., 2009; Wolter et al., 2014). On the contrary, the *L. amylovorus* sourdough showed the optimum addition level at 18% which translates to about 6 mmol of lactic acid per 100 g of bread dough. Addition levels of 6% (ca. 2 mmol lactic acid per 100 g) and 12% (ca. 4 mmol of lactic acid per 100 g) did not influence the volume significantly and the reduction of loaf volume found at the highest addition level of 24% (ca. 9 mmol of lactic acid per 100 g) can be traced back to the high amount of acid in the bread dough. The type of acids and the acid concentration are the factors determining whether the impact on bread quality is beneficial or contra-productive (Wehrle et al., 1997). This effect is based on the technological impact of acids on the baking process (Arendt et al., 2007; Moore et al., 2008). While small amounts of acid are known to improve bread quality characteristics such as specific volume, higher additions of sourdough and therefore, higher acid concentrations, reduce the specific volume but improve freshness and shelf-life (Komlenic et al., 2010; Rozylo et al., 2015a, 2015b). As the *W. cibaria* sourdough is only a low acidified sourdough, the acid addition had for all addition levels positive impact on the loaf volume in combination with the known beneficial impact of EPS on yeasted wheat bread (Galle et al., 2010; Katina et al., 2009; Wolter et al., 2014).

The total numbers of cells per slice did not differ between the samples. Only for the highest addition level of *L. amylovorus* sourdough the cell number was significantly higher, with an average of 4142 ± 118 cells per slice. By increasing the amounts of *W. cibaria* sourdough addition, porosity, wall thickness, cell/loaf volume also increased (Table 4).

The combination of 6% of *L. amylovorus* sourdoughs and 18% of *W. cibaria* sourdough in the bread formulation resulted in a loaf volume and bread crumb characteristics which were not significantly different to the sample containing 18% *W. cibaria* sourdough. The low addition

Table 5
Mean panel description sensory score for the attributes determined by the descriptive sensory categorised into the four main sensory responses: appearance (A), texture (T), olfactory (O) and flavour (F).

Product	Appearance	Texture response	Olfactory responses				Flavour responses					
	A density	T density	O roasted	O sourdough	O cheesy	O doughy	O yeasty	F yeast	F doughy	F musty/stale	F roasted	Salt	Bitter
Control 0.0% NaCl	$60.5^{e,f}$	$48.7^{e,f}$	$4.4^{a,b}$	$13.9^{b,c}$	0.00^{a}	$31.3^{b,c,d}$	$26.8^{a,b}$	$29.0^{a,b,c,d}$	$28.2^{a,b,c}$	$8.5^{a,b,c}$	0.1^{a}	5.6^{a}	$2.6^{b,c}$
Control 0.3% NaCl	$9.6^{c,f}$	$45.5^{d,e,f}$	$4.9^{a,b}$	$9.7^{a,b}$	0.75^{a}	$28.6^{a,b,c,d}$	$29.9^{a,b,c}$	$26.5^{a,b,c,d}$	$33.1^{c,d}$	$6.6^{a,b,c}$	0.3^{a}	7.6^{a}	$1.8^{a,b,c}$
Control 1.2% NaCl	83.8^{f}	70.7^{g}	$2.3^{a,b}$	$12.0^{a,b}$	0.04^{a}	45.4^{e}	21.3^{a}	21.6^{a}	44.4^{e}	18.2^{d}	0.2^{a}	58.4^{b}	$1.4^{a,b}$
6% *L. amylovorus* 0.0% NaCl	$44.3^{a,b,c,d,e}$	$35.3^{b,c,d,e}$	$16.6^{b,c}$	$7.7^{a,b}$	15.88^{c}	16.1^{a}	$29.9^{a,b,c}$	$28.8^{a,b,c,d}$	$23.0^{a,b}$	$6.1^{a,b,c}$	$3.4^{a,b,c}$	4.1^{a}	0.5^{a}
6% *L. amylovorus* 0.3% NaCl	$58.9^{e,f}$	$45.3^{d,e,f}$	$17.1^{b,c}$	$9.4^{a,b}$	7.80^{b}	$21.3^{b,c}$	$28.1^{a,b,c}$	$24.5^{a,b}$	$27.6^{a,b,c}$	$6.9^{a,b,c}$	$3.3^{a,b,c}$	9.3^{a}	$2.0^{b,c}$
6% *L. amylovorus* 1.2% NaCl	$68.7^{e,f}$	$59.8^{f,g}$	$1.3^{a,b}$	19.7^{c}	5.06^{b}	$38.1^{d,e}$	21.1^{a}	$23.7^{a,b}$	$39.2^{d,e}$	11.4^{c}	0.2^{a}	$63.8^{b,c}$	$1.4^{a,b}$
18% *W. cibaria* 0% NaCl	$26.0^{a,b}$	$25.0^{a,b,c}$	$11.7^{a,b,c}$	6.1^{a}	0.03^{a}	16.1^{a}	$41.0^{d,e}$	$36.4^{d,e}$	$28.0^{a,b,c}$	$6.3^{a,b,c}$	$1.2^{a,b}$	5.2^{a}	3.1^{c}
18% *W. cibaria* 0.3% NaCl	$52.0^{c,d,e}$	$45.1^{d,e,f}$	$4.5^{a,b}$	$11.0^{a,b}$	0.03^{a}	$31.9^{c,d,e}$	$29.6^{a,b,c}$	$30.1^{a,b,c,d,e}$	$30.7^{a,b,c,d}$	$10.7^{b,c}$	0.2^{a}	12.5^{a}	$1.1^{a,b}$
18% *W. cibaria* 1.2% NaCl	$57.6^{d,e}$	$51.9^{e,f}$	$5.9^{a,b}$	$9.7^{a,b}$	0.00^{a}	$33.2^{c,d,e}$	$33.2^{b,c,d,e}$	$30.2^{a,b,c,d,e}$	$32.3^{b,c,d}$	11.5^{c}	$1.2^{a,b}$	$64.2^{b,c}$	$1.3^{a,b}$
18% *W. cibaria* + 6% *L. amylovorus* 0.0% NaCl	18.6^{a}	15.9^{a}	$16.7^{b,c}$	$10.6^{a,b}$	15.16^{c}	16.0^{a}	44.0^{e}	38.7^{e}	21.9^{a}	3.3^{a}	$1.5^{a,b,c}$	3.6^{a}	3.2^{c}
18% *W. cibaria* + 6% *L. amylovorus* 0.3% NaCl	$33.8^{a,b,c,d}$	$28.1^{a,b,c,d}$	33.5^{d}	6.1^{a}	7.34^{b}	$19.3^{a,b,c}$	$42.1^{d,e}$	$34.6^{c,d,e}$	$28.5^{a,b,c}$	$6.3^{a,b,c}$	$4.0^{b,c}$	12.3^{a}	$1.9^{b,c}$
p value	0.0012	0.0002	0.0013	0.0293	0.0000	0.0028	0.0025	0.0123	0.0029	0.0043	0.0398	0.0000	0.0370

Values followed by a different letter are significantly different ($p < 0.05$).

level of 6% *L. amylovorus* sourdough did not impact on the loaf volume or the crumb quality as previously determined for the same addition level to the low-salt control.

Crumb hardness was measured over a storage period of five days. NaCl reduction resulted in a softer crumb after two and five days of storage but no difference could be detected on the baking day (Fig. 2). When sourdough was added at 24%, the network connectivity significantly differed, impacting on dough network. For the addition of 24% *W. cibaria* sourdough (=11 mg EPS per 100 g bread dough) the bread after 5 days storage was as soft as the control breads on the baking day. Similar effects were reported by Wolter et al. (2014) and (Galle et al., 2012) who could show a significant increase of crumb softness as well as significantly reduced staling rates in breads made out of different flours containing 20% of the *W. cibaria* sourdough. The addition of *L. amylovorus* sourdough increased the crumb hardness only for the maximum addition level of 24% having the highest staling rate of all breads. The low-salt control and the *L. amylovorus* sample at 6% addition level showed no difference in staling over the five days of storage. With increasing addition levels of *W. cibaria* sourdough the staling was delayed and the bread crumb became softer. The *W. cibaria* sample with 24% sourdough resulted in the softest crumb followed by the sample with an additional level of 18% sourdough which had also the optimal specific bread volume. The delayed staling and softer crumb was most likely based on different speed in starch crystallisation by EPS and not by the increased loaf volume (Davidou et al., 1996; Galle et al., 2012). For the combination of both sourdoughs the crumb hardness and staling behaviour were not significantly different to the *W. cibaria* samples with addition level of 12% and 18%. The small amount of organic acid added with the *L. amylovorus* sourdough could be compensated with the presence of EPS and hence, has no negative influence on crumb hardness.

3.5. Microbial shelf-life and water activity

The microbial shelf life of all bread samples was assessed exposing the different samples of bread slices to the bakery environment for a defined time of 10 min. The reduction of NaCl in bread without sourdough addition from standard salt to low-salt resulted in a reduced shelf life of about two days as previously reported (Belz et al., 2012a, 2012b; Samapundo et al., 2010). The loss of shelf life of two days based on NaCl reduction could be compensated with the addition of 6% sourdough containing the antifungal strain *L. amylovorus* DSM19280 to low-salt bread. Each additional 6% of sourdough extended the shelf life by a further two days resulting in approx. 13 days for 24% sourdough addition. On the contrary, the addition of sourdough fermented with *W. cibaria* MG1 did not result in a significant change of the shelf life for low-salt breads (Fig. 3). These results correlate with the fact that the *W. cibaria* bacteria are a low acidifying strain and an insignificant amount of antifungal acid compounds could be detected (Fig. 1). The combination of both sourdoughs resulted in a shelf life of 6–7 days which was based on the antifungal effect of the addition of 6% *L. amylovorus* sourdough. The additional 18% *W. cibaria* sourdough had no influence on the microbial bread shelf life.

The water activity of the bread crumb samples was measured and resulted in a direct correlation to the added amounts of sourdough (Fig. 4). The sourdough fermented with *L. amylovorus* DSM19280 lead to a lower a_w compared to *W. cibaria* but only at the highest addition level of 24% sourdough the a_w was significantly different with $a_w = 0.982 \pm 0.002$ for *W. cibaria* MG1 compared to 0.979 ± 0.002 for *L. amylovorus* DSM 19280. The water binding nature of EPS resulted in a higher water activity for the bread samples containing *W. cibaria* MG1. None of the sourdough breads with 0.3% NaCl reached the water activity of the standard salt bread of $a_w = 0.974 \pm 0.002$. The fact that the water activity did not correlate with the determined shelf life can be explained with the antifungal compounds found in the sourdough fermented with the *L. amylovorus* DSM19280 (Fig. 1).

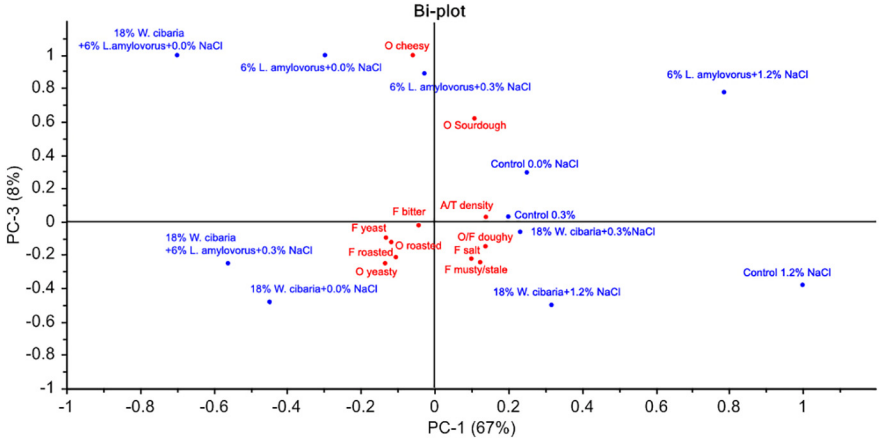

Fig. 5. Principle component analysis of sensory attributes of bread samples containing different amounts of NaCl (1.2%; 0.3%; 0.0%) and different sourdoughs (6% sourdough fermented with *L. amylovorus* and 18% sourdough fermented with *W. cibaria*). O = odour; F = flavour; A = appearance; T = texture.

3.6. Sensory evaluation

The set of breads selected for the descriptive sensory analysis section of the research was based on the best performance of the quality characteristics of the bread loaves. For the samples containing *L. amylovorus,* a level of 6% was chosen, based on the shelf life of 6 days for the low salt level of 0.3% NaCl. The samples containing *W. cibaria* resulted in the best quality loaves with respect to volume and crumb structure at an addition of 18%, and was also chosen for the descriptive sensory analyses. While a level of 24% additional sourdough resulted in an even higher bread loaf volume, the crumb texture of this loaf was too porous and open to be chosen. Furthermore, for comparison reasons three more control samples were added without any NaCl.

Table 5 shows the average panel scores for the sensory characteristics measured. The assessors were able to differentiate between the samples for all attributes measured. Increasing amounts of NaCl let to a higher bread crumb density independent of the addition of sourdough. This was found for both appearance and texture density. Based on the inhibition of yeast activity by NaCl the increased density was caused by a reduced gas production by the baker's yeast during proofing (Lynch et al., 2009). For the olfactory attributes “roasted” and “sourdough” no significant impact could be determined. The aroma note “cheesy” was significantly more intense adding the *L. amylovorus* sourdough. A reduced NaCl concentration resulted in an additional significantly higher cheesy aroma. The PCA generated from all the attributes measured was used to summarize the relationship between the samples and these attributes, allowing these to be visualized easily. PC1 accounted for 67% of the determined variances and it distinguished the samples mainly between a “yeasty” flavour and odour on the one side and a “salty” and musty/stale flavour on the other side. PC2 separated the samples mainly between “sour” and “bitter” flavours which were not relevant for the presented work. Across PC3 which accounts for 8% the samples were split between a “cheesy, sourdough” like flavour and a “salty” flavour. The PCA (Fig. 5) was generated using PC1 and PC3. Hence, most samples with a NaCl concentration of 1.2% can be found in the right bottom quadrant. The one exception is the sample also containing 6% of the *L. amylovorus* sourdough, which significantly showed a cheesy/sourdough like note independent of the NaCl concentration (Table 5). The samples were distinguished across the PC3 (Fig. 5) between “cheesy” and “salty” which shows that both attributes compete against each other. The yeasty attributes were influenced mostly by the NaCl concentration resulting in an increased yeast perception for reducing amounts of NaCl. This correlates again with the descript yeast activity being influenced by NaCl. The reverse effect was determined for the doughy attributes. The higher the NaCl concentration the higher the doughy perception. Salt is known to enhance other flavours (Gillette, 1985; Kare, 2012) resulting in a higher recognition of the doughy compounds originating from the wheat flour in combination with water and yeast. Independent of the use of sourdough, no difference of the perception of salt was recognised between 0.0% and 0.3% NaCl but significantly different for the standard amount of 1.2%. For the omission of salt the assessors determined a significant increase of bitterness. As previously reported sodium suppresses bitterness (Breslin and Beauchamp, 1995a, 1995b) enhancing other flavours (Breslin and Beauchamp, 1997). However, the addition of sourdough avoided an increase of bitterness.

Hence, all samples containing *L. amylovorus* sourdough are located in the upper half showing the typical cheesy note of this particular sourdough. The attributes “density” correlate with the determined specific volumes of the bread loaves. The addition of sourdough fermented with *W. cibaria* increased the volume significantly. As previously reported, a more porous crumb with lower density leads to a more intense recognition of salt and possibly of other flavours too (Konitzer et al., 2013; Pflaum et al., 2013). Both NaCl containing samples with 18% *W. cibaria* sourdough (1.2% and 0.3% NaCl) were described as slightly saltier than the respective control samples with 1.2% and 0.3% NaCl. Hence, the increased loaf volume, caused by the added EPS with the *W. cibaria* sourdough, resulted in a coarser crumb texture and thus, the salt perception was more intense for the assessors compared to the control breads. In addition, the lactic and acetic acids enhanced the saltiness perception of the bread crumb (Reddy and Marth, 1991). The combination of both sourdoughs led for 0.0% NaCl to the sample described as most “yeasty” and “cheesy”. The increased loaf volume enhanced the cheesy flavour of the *L. amylovorus* sourdough and increased the perception. The addition of 0.3% NaCl changed the flavour profile significantly away from the cheesy flavour towards a more salty one.

4. Conclusion

The use of *L. amylovorus* DSM19280 had previously been shown to prolong the microbial shelf life significantly (Axel et al., 2015; Belz et al., 2012a, 2012b). The present study determined the minimum amount of 6% sourdough addition to compensate the reduced shelf life caused by the salt reduction. The antifungal effect was mainly based on the determined antifungal compounds 3-phenyllactic acid, 4-hydroxyphenyllactic acid, leucic acid and azelaic acid. The shelf life of a low salt bread of about 4 days could be prolonged to the same shelf life of a standard salt bread of about 6 days. The EPS producing strain *W. cibaria* MG1 was found to increase the bread volume and the bread crumb porosity as well as delaying the bread staling significantly with an optimum addition level of 18%. The descriptive sensory analyses

distinguished mainly between a more “roasted” and “yeasty” versus a more “salty” and “dough” perception. The sourdough started with *L. amylovorus* DSM19280 and *W. cibaria* MG1 lead to a low-salt bread with improved shelf life, a softer bread crumb, increased bread volume and an improved sensory profile.

The presented work could demonstrate that the use of functional sourdoughs can compensate the amount of salt reduced in low-salt bread. High product quality for low-salt bread can be achieved in a natural way without any additives, matching standard salt bread closely. Considering reports about adaptation of the saltiness perception based on a gradual reduction of salt in food, the achieved result would be a natural alternative for the long-term goal of a low-salt bread.

Acknowledgement

The authors would like to thank Tom Hannon for his technical support and Sandra Galle and Alice Moroni for advice. Furthermore, the authors wish to acknowledge that this project was funded under the Irish National Development Plan, through the Food Institutional Research Measure, administered by the Department of Agriculture, Fisheries and Food, Ireland 06RDC455.

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