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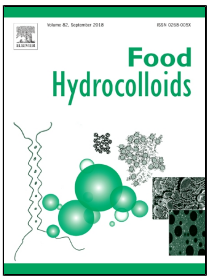

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Dextran produced *in situ* as a tool to improve the quality of wheat-faba bean composite bread

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Abbreviations:

ANF, anti-nutritional factors; CA, chemically acidified; κ-CAR, Kappa carrageenan; CFU, colony-forming unit; CMC, carboxymethyl cellulose; DY, dough yield; EPS, exopolysaccharides; FAA, free amino acids; FQ, fermentation quotient; FSB, faba bean sourdough bread; FWB, faba bean wheat bread; GG, guar gum; HPMC, hydroxypropylmethyl cellulose; LAB, lactic acid bacteria; Mw, molecular weight; SD, sourdough; TPA, texture profile analysis; TTA, total titratable acidity.

Abstract

The incorporation of faba bean flour into wheat-based products is a sustainable way to obtain protein-enriched food items. However, developing breads with a higher content of faba bean flour is challenging due to the poor textural/sensory properties of the final product. A potential solution is to use hydrocolloids as structuring agents to increase the viscoelastic properties of the composite bread. Microbial dextran is a natural hydrocolloid which can be used as a bread texture improver either as a pure food ingredient or by *in situ* production during sourdough fermentation. The aim of this study was to compare the influence of dextran produced *in situ* by *Weissella confusa* VTT E-143403 (E3403) and *Leuconostoc pseudomesenteroides* DSM 20193 in faba bean sourdoughs on the quality of wheat bread supplemented with 43% faba bean sourdough. The impact of dextran on the rheological properties of dough and textural properties of the final bread were evaluated. Dextran formed by *W. confusa* and *L. pseudomesenteroides* reached a level of 5.2 and 3.6% (flour basis), respectively. Incorporation of faba bean sourdough containing dextran synthesized by *W. confusa* improved the dough viscoelastic properties, and also increased the specific volume (~21%) and reduced crumb hardness (~12%) of the final bread, compared to control breads. Similar positive effects were not obtained with sourdough containing dextran from *L. pseudomesenteroides*, probably due to its higher acidity. Dextran synthesized *in situ* by *W. confusa* is a promising clean label hydrocolloid option to improve the quality of wheat bread enriched with faba bean flour.

Keywords

Dextran; Hydrocolloids; Rheology; Faba bean; Fermentation; Bread

1. Introduction

Faba bean (*Vicia faba* L.) is one of the oldest crops in the world and has a high content of good-quality proteins (~30% of proteins with high lysine content), fiber, vitamins, minerals and bioactive substances such as phenols and γ-aminobutyric acid (Jezierny, Mosenthin, & Bauer, 2010). In recent decades, several studies have examined the incorporation of faba bean flour into wheat-based food to produce nutritionally improved products such as pasta (Giménez et al., 2012; Rizzello et al., 2017; Rosa-Sibakov et al., 2016) and bread (Abdel-Kader, 2000). Substitution with faba bean flour might represent a more sustainable and economical way to improve bread protein content (Coda, Varis, Verni, Rizzello, & Katina, 2017b) and compensate for the essential amino acid deficiencies (lysine and threonine) in wheat (Abdel-Aal & Hucl, 2002).

However, the use of faba bean in food applications is restricted due to the presence of anti-nutritional factors (ANF), such as enzyme inhibitors, phytates, condensed tannins and galactooligosaccharides (Jamalian, 1999) and its undesirable beany flavor. Another major limiting factor of faba bean use in bread making is the poor baking performance of its proteins compared to wheat gluten. Generally, substituting wheat flour with 10% or more of faba bean ingredients is a challenge to the baking process. Dilution of the gluten matrix reduces the stability of the dough, giving the bread a lower volume and harder texture (Pérez, Ribotta, Steffolani, & León, 2008). Additionally, replacing wheat with faba bean flour could lead to changes in starch gelatinization and cell wall thickness of the crumb (Ferrero, 2017).

A potential approach to increase the wheat-faba bean flour bread making functionality is to use hydrocolloids to mimic the viscoelastic and gas binding properties of gluten. Hydrocolloids, in particular HPMC (chemically modified) and dextran (natural), are shown to increase the bread volume and decrease crumb firmness (Guarda, Rosell, Benedito, & Galotto, 2004; Rosell, Rojas, & Benedito de Barber, 2001; Zannini, Waters, & Arendt, 2014). The positive effects of hydrocolloids on dough systems and breads

65 are associated with two main techno-functionalities: (1) the water binding ability and the modification
66 of water distribution and (2) interactions with dough structural components such as gluten, non-gluten
67 proteins, and starch. Hydrocolloids are proposed to stabilize the interface of the dough liquid film
68 surrounding gas bubbles, thus prevent collapsing and improve gas retention (Bárcenas & Rosell, 2005).
69 Dextran, which is a novel food ingredient, has been approved by the European Commission for utilization
70 in bakery products with the claim that dextran produced by bacterial fermentation at an addition level
71 below 5% (end product basis) in bakery products, does not constitute a safety concern for consumer
72 health (18/10/2000). However, dextran is not widely used compared to HPMC. This might be attributed
73 to its novel status and limited studies on its baking performance. Also, its relatively higher-cost of
74 production possibly restricts its use as a food additive (De Vuyst & De Vin, 2007). Previously, dextran
75 has been introduced to bread applications in two ways: either the as a purified ingredient or by *in situ*
76 production during fermentation with selected lactic acid bacteria (LAB). The addition of dextran with
77 specific characteristics, able to improve volume, crumb structure, softness and mouthfeel of bread could
78 be more straightforward than the *in situ* production. However, this approach is currently restricted by
79 several factors, including the type of dextran to be used and the high costs. Moreover, the *in situ*
80 production is accompanied by many metabolic beneficial effects, and is recognized as a clean label
81 approach meeting consumer demand for no/reduced food additives.

82 Studies are however required in order to optimize the performance of *in situ* produced dextran. The
83 chemical structure of the dextran produced, production efficiency and the metabolic traits of the producer
84 LAB such as acidification, are important considerations. Dextrans are exopolysaccharides (EPS)
85 synthesized from sucrose by extracellular enzymes dextransucrases produced by LAB of the genera
86 *Weissella, Leuconostoc*, *Streptococcus*, *Pediococcus* and *Lactobacillus* (De Vuyst & De Vin, 2007).
87 They are α-glucans, which contain α-(1→6)-linked D-glucopyranosyl units in the main chain with

variable amounts of α-(1→2)-, α-(1→3)-, or α-(1→4)-branched linkages (Monsan et al., 2001). The variations in the type and degree of branching, length of branched chains, and molecular weight (Mw) of dextrans depend on the strain and thereby on the dextransucrases that it expresses. Generally, the effect of dextrans on the dough rheology and textural properties of the bread depends on their molecular weight (Mw), linkage type, degree of branching and conformation. Dextrans with high Mw and α-(1→3)-linked branching (3-9%) have been found to increase the water–binding capacity and inhibit staling of bread, which promote superior structural effects in both wheat and gluten-free breads (Rühmkorf et al., 2012; Zhang et al., 2018). These dextrans are suggested to create a structured polysaccharide network stabilized by hydrogen bonds or steric interactions, which aid the gluten network and increase gas retention (Ross, McMaster, David Tomlinson, & Cheetham, 1992). Furthermore, the high Mw dextrans retard the formation of amylopectin crystallites and thus delay bread staling (Zhang et al., 2018). In contrast, dextran with a lower Mw has been shown to decrease the water absorption and bread loaf volume, which has been suggested to interfere with optimal gluten network formation (Ross et al., 1992). Successful application of dextran can be affected by the organic acids that are simultaneously produced during LAB fermentation. Adding sucrose not only enables the production of dextran but also affects the formation of acetic acid. In obligate heterofermentative LAB like *Leuconostoc* spp., fructose released from sucrose is metabolized to mannitol and acetate in a molar ratio of 2:1 (Erten, 1998). High levels of acetate formation following sucrose addition can counteract the positive technological effects of dextran formed *in situ*. Studies on cereal flours show that *Weissella* spp. strains are able to produce a considerable amount of dextran *in situ* but typically do not form excess acetate due to the lack of mannitol dehydrogenase (Schwab, Mastrangelo, Corsetti, & Gänzle, 2008).

The aim of this research was to study the influence of *in situ* dextran production in faba bean sourdough and its technological performance in composite dough and bread containing wheat and 30% fermented

faba bean flour. The study employed two strains, *Weissella confusa* E3403 and *Leuconostoc pseudomesenteroides* DSM 20193, with different fermentation profiles. Their effect on the rheological properties of the dough and quality of the composite breads was compared.

2. Materials and methods

2.1 Materials

The materials used included faba bean flour (San Martino di Lupari, PD, Italy; protein 30.0%, fat 1.5%, fiber 16.0%, moisture 12.0%), wheat flour (Fazer Mill & Mixes, Finland; protein 14.0%, fat 1.7%, fiber 5.3%, moisture 14.0%), fresh yeast (Lallemand, Lahti, Finland), sucrose (Dansukker, Finland) and salt (Helsinki, Finland).

2.2 LAB strains and growth conditions

Dextran-forming *Weissella confusa* VTT E-143403 (E3403) was obtained from the VTT Culture Collection and *Leuconostoc pseudomesenteroides* DSM 20193 was purchased from the Leibniz Institute DSMZ (Braunschweig, Germany). The strains were routinely cultivated in MRS broth (Oxoid, Basingstoke, UK) at 30°C for 24 h. For preparation of sourdough, strains were subcultured in general edible medium (GEM, 20 g dextrose, 20 g sucrose, 30 g soy peptone, 7 g yeast extract, 1 g $MgSO_4 \cdot 7H_2O$ in 1 L 0.01M potassium phosphate buffer, pH 6.3).

2.3 Sourdough preparation

Faba bean flour and distilled water were mixed with a mixer (Robert Bosch GmbH, Germany) to a dough yield of 250 (DY = 100 × [(g flour + g water)/g flour]) (Table 1). Microbial cells were obtained from cultures incubated overnight through centrifugation (12,000g, 15min), washed once with sodium phosphate buffer saline (PBS, 8.2 g NaCl, 1.7g $K_2HPO_4 \cdot 3H_2O$, 0.2 g KH_2PO_4 in 1 L Milli-Q water, pH 7.4) and inoculated into the sourdoughs at an initial cell density of 10^6 CFU (colony forming units) g^{-1}.

For *in situ* formation of dextran, 10% (w/w) of faba bean flour was substituted with sucrose (EPS-positive sourdough). EPS-negative sourdoughs were prepared with the same starters but without sucrose supplementation. The sourdoughs were fermented for 24 h at 25°C. Chemically acidified control (CA) doughs were prepared with comparable amounts of lactic and acetic acid and to the same final pH and total titratable acidity (TTA) as measured in the fermented *W. confusa* E3403 and *L. pseudomesenteroides* DSM 20193 sourdoughs with sucrose addition. The CA doughs were incubated at 25°C for 1 h before bread making or dough rheology analysis.

2.4 Determination of cell counts, pH and TTA in sourdough

Cell counts of LAB were determined at fermentation time 0 and 24 h by serial dilutions in sterile saline solution and subsequent plating on MRS agar (Lab M, Heywood, UK). The plates were incubated for 48 h at 30°C in anaerobic conditions. Total mesophilic bacteria were determined on Plate Count Agar (PCA, Lab M) under aerobic conditions at 30 °C for 48 h. The pH values of the sourdough samples were measured at 0 and 24 h using a pH meter (Model HI 99161, Hanna Instruments, Woonsocket, RI, USA). The acidity (pH and TTA) of the sourdough samples was determined as the amount of 0.1 M NaOH required to adjust the end pH of 10 g samples in 100 ml Milli-Q water to 8.5, as described elsewhere (Katina, Salmenkallio-Marttila, Partanen, Forssell, & Autio, 2006). All measurements were done in triplicate.

2.5 Sourdough viscosity

A rotational rheometer (Rheolab QC, Anton Paar, Germany) was used to measure the viscosities of the sourdoughs before and after fermentation at 20°C. The measuring profile included shear rates from 2 s^{-1} to 300 s^{-1} and back to 2 s^{-1}. The measurements were done in triplicate.

2.6 Determination of free sugars and organic acids in sourdough

For analysis of metabolites, 10 g of sourdough was dissolved in 100 ml of distilled water and homogenized with a Bamix blender for 60 s at room temperature. To ensure removal of the apolar component and proteins, 1.5 ml ethanol and 3 ml 30% (w/v) Na_2SO_4 were added. Samples were further diluted with distilled water to a constant volume of 200 ml and incubated overnight at 4°C. After centrifugation at 12,000 g for 15 min, supernatant was collected and filtered with a 0.2 µm nylon membrane. Quantification of sucrose, glucose and fructose in the extracts was done with a commercial K.SUFRG (Megazyme, Wicklow, Ireland) enzymatic kit. The content of lactic acid and acetic acid in the extracts was determined with other commercial kits, K-DLATE and K-ACET (Megazyme). All analyses were performed in triplicate.

2.7 Analysis of dextran in sourdough

The dextran formed in the sourdoughs was extracted with an enzyme-assisted method according to Katina et al. (Katina et al., 2009). The amount of dextran was determined with high performance anion exchange chromatography with pulse amperometric detection (HPAEC-PAD). The HPAEC-PAD system contains an analytical CarbPac PA-1 column (250 × 4 mm, i.d, Dionex, Sunnyvale, CA, USA), a Waters 2465 pulsed amperometric detector (Waters, Milford, MA, USA), a Waters 2707 autosampler, and three Waters 515 HPLC pumps. The eluents used were Milli-Q water and 200 mmol l^{-1} NaOH as the mobile phase at a flow rate of 1.0 ml min^{-1}. Glucose (Merck, Germany) was used as the external standard and 2-deoxy-galactose as the internal standard for quantification.

2.8 Baking procedure

The recipes for wheat breads (WB), faba bean-wheat composite breads without sourdough (FWB), faba bean-sourdough composite breads (FSB) and chemically acidified composite breads (CAB) are described in Table 1. Breads without sourdough (WB, FWB and CAB) were used as controls. Wheat flour

substituted with faba bean flour at a level of 30% w/w (=43% faba bean sourdough) was used in bread making. The substitution level (30%) of faba bean flour was determined based on calculation (nutritional composition) to obtain a 20% protein content of the total energy value (Table 2 and 3). The regulations of the European Parliament and Council (20/12/2006) propose that the nutrition claim 'high in protein' be allowed for food products when 20% of the energy value is provided by protein. The optimal water content for the breads was based on wheat flour as determined with a Brabender Farinograph (Brabender GmbH & Co.KG, Germany), according to AACC method 54-21 (AACC 2000). For all breads, the total amount of water (including in sourdough where applicable) was the same: 63% v/w of the total flour weight with a dough yield of 163. Breads were prepared by mixing all the ingredients in a DIOSNA mixer bowl (Dierks & Söhne GmbH, Germany) for 3 min at low speed and 4 min at fast speed. After 15 min proofing in a fermentation cabinet (Lillnord, Odder, Denmark) at 35°C and relative humidity (RH) of 75%, the dough was divided into pieces of 250 g. The doughs were molded mechanically and rested in pans for 45 min (35°C, RH 75%). The breads were baked in a rotating rack oven (Sveba Dahlen, Fristad, Sweden) at 220°C for 15 min with 15 s steaming at the beginning. After baking, the loaves were depanned and cooled for 1 h at room temperature before weighing. Baking was done on two different days (two independent baking trails) and nine breads were prepared for each bread type. The breads were stored in plastic bags overnight and the loaf volume determined with the rapeseed displacement method. The specific volume of the bread was calculated by dividing the loaf volume (mL) by the weight (g). Texture Profile Analysis (TPA) of bread crumbs was done with a texture analyzer (TA, TA-XT2i, Stable Micro Systems Ltd., UK) using a 25-mm diameter aluminum probe on days 1 and 4 of storage as described elsewhere (Katina, Heinio, Autio, & Poutanen, 2006). Samples for testing were prepared by cutting into 50 mm x 50 mm x 25 mm slices and the edges were removed. The TPA results were calculated based on the percentage of the FWB control bread due to variations in the baking dates.

200 *2.9 Measuring of pH and TTA in bread crumb*

201 For determining the acidity of the bread, the crust of the slice was removed and the crumb (10 g) was
202 homogenized with 5 ml of acetone and 95 ml of Milli-Q water using a Bamix blender. The PH and TTA
203 of the suspension were measured as described above.

204 *2.10 Dough rheology*

205 *2.10.1 Farinograph mixing characteristics*

206 Farinograph was determined using a 50 g mixing bowl with the same optimal water content as for the
207 control wheat flour (63% v/w) according to the AACC method. The doughs were prepared as described
208 for baking without yeast and with salt addition to ensure reproducibility of the measurements, since gas
209 bubbles generated by yeast fermentation would affect the rheological properties. Two parameters were
210 determined from the farinograms: water absorption (WA)—percentage of water required to obtain a
211 standard dough consistency of 500 BU and the maximum dough consistency (consistency at the peak of
212 the curve).

213 *2.10.2 SMS/Kieffer dough and gluten extensibility rig*

214 For extensibility measurement, the doughs were mixed to optimal dough development for 6 min in the
215 Farinograph. After mixing, ~15 g of dough was used for extension measurement as previously published
216 (Smewing, 1995), with a few modifications to the resting times and temperature. Dough extensibility
217 measurement was carried out with a SMS/Kieffer dough and gluten extensibility rig on a TA-XT2i
218 texture analyzer with a 5 kg load cell and a Plexiglas cabinet to maintain the testing temperature. The
219 dough was rested in the fermentation cabinet (35°C, RH 75%) for 20 min. After relaxing, the dough was
220 molded manually, first into a ball and then into a cylinder. A Teflon form was greased with paraffin oil
221 and preheated at 35°C for 1 h with lametta strips laid in the grooved base. The cylinder-shaped dough

222 was pressed into the form, covered with a plastic bag, and allowed to rest in the fermentation cabinet for
223 40 min. The test pieces were removed with a spatula, lifting the strips without deforming them. The test
224 speed was 2.0 mm/s and distance 85 mm. The hook probe of the Kieffer rig stretched the dough centrally
225 until rupture. The peak force (maximum resistance to extension (R_{max})), extensibility (total length of the
226 curve (Ext)), and strength (total area under the dough extensibility curve (A_{tot})) were recorded for five
227 dough strips per dough.

228 *2.10.3 Fundamental rheology*

229 The mixed dough sample from the Farinograph was allowed to relax for 30 min at room temperature and
230 then used for oscillatory measurements. Frequency sweep tests were performed on a Haake RheoStress
231 rheometer (RS 50, Haake Rheometer, Karlsruhe, Germany) with a parallel plate geometry (diameter 35
232 mm, gap 2.5 mm). The samples (3.3 g) were molded by hand into a ball and placed in the center of the
233 plate. Water drops were placed around the bottom plate to prevent contact with the dough sample and
234 the sample covered with a hood to avoid moisture loss. The tests were performed at a constant
235 temperature of 20°C. Before oscillatory measurement, the samples were allowed to rest for 5 min and
236 the linear viscoelastic region was determined using the amplitude sweeps. Frequency sweeps were
237 performed at 0.05 to 10 Hz. All tests were done in triplicate and the averages calculated.

238 *2.11 Statistical analysis*

239 Statistical analysis was performed with one-way univariate analysis of variance (ANOVA) using IBM
240 SPSS Statistics 23 (IBM SPSS Inc., United States) on all data with Turkey's test (significance level P
241 <5%).

242 **3. Results**

243 *3.1 Growth, pH and TTA of sourdoughs and bread crumb*

The initial cell count of total mesophilic bacteria and lactic acid bacteria in all sourdoughs was approximately 10^6 cfu/g (Table 4). After 24 h of fermentation, the cell densities of presumptive lactic acid bacteria in the sourdoughs fermented by *L. pseudomesenteroides* DSM 20193 and *W. confusa* E3403 reached 9.8 and 9.5 log cfu/g, respectively. No significant difference was observed between EPS-positive and EPS-negative sourdoughs. After fermentation, the mesophilic bacteria count showed very similar values between the sourdoughs and was comparable to the cell densities of lactic acid bacteria ($p > 0.05$).

In general, significantly lower pH values and correspondingly higher TTA values were reached in *L. pseudomesenteroides* sourdoughs than in *W. confusa* sourdoughs (Table 4). The pH value measured in *L. pseudomesenteroides* EPS-positive sourdough (with sucrose addition) after fermentation was 4.6, and a similar pH was found in the EPS-negative counterpart (without sucrose addition). The pH value measured in *W. confusa* EPS-positive sourdough was 5.1 but significantly higher in its EPS-negative counterpart (5.6). Regardless of the strain used, the addition of sucrose in sourdoughs resulted in a significant increase of TTA values compared to sourdoughs without added sucrose. Similarly, lower pH values and higher TTA values were measured in *L. pseudomesenteroides* FSB than in the *W. confusa* FSB. FSB showed significantly lower pH values and at the same time higher TTA values compared to the controls (FWB and WB).

3.2 Free sugar and organic acid formation in sourdoughs

Sucrose utilization during sourdough fermentation was confirmed by determination of the amount of extractable free sugars. The added sucrose (10% of the flour weight) was completely consumed by *L. pseudomesenteroides* and *W. confusa* (Table 5). A significant amount of fructose (4.6% of the flour weight) was detected in EPS-positive *W. confusa* sourdoughs but not in *L. pseudomesenteroides* EPS-positive sourdoughs (1.5%).

The amount of acetic acid in EPS-positive *L. pseudomesenteroides* sourdough (2.8 g/kg SD) was double that in the EPS-negative counterpart (1.2 g/kg SD). In contrast, similar amounts of lactic acid (3.6 g/kg and 3.2 g/kg SD) and acetic acid (1.3 g/kg and 1.4 g/kg SD) were formed in both EPS-positive and EPS-negative *W. confusa* sourdough, respectively. The fermentation quotient (FQ), or molar ratio between lactic acid and acetic acid, increased in the following order: EPS-positive L. *pseudomesenteroides* (0.9) < EPS-negative *W. confusa* (1.5) < EPS-positive *W. confusa* (1.8) < EPS-negative L. *pseudomesenteroides* sourdough (2.6).

3.3 Dextran formation and its effect on sourdough viscosity

The viscosity was analyzed to show the influence of dextran and acid production on the rheological properties of the sourdoughs (Figure 1). All the sourdoughs displayed a shear thinning behavior. Increased viscosity of the sourdoughs after fermentation was observed in all the sourdoughs compared to the non-fermented control sourdough. Both *L. pseudomesenteroides* and *W. confusa* EPS-positive sourdoughs exhibited significantly higher viscosities than their EPS-negative counterparts. The EPS-positive *W. confusa* sourdough had the highest viscosity, indicating that *W. confusa* synthesized a significant amount of dextran with an impact on dough viscosity.

Dextran formation by *L. pseudomesenteroides* and *W. confusa* in sourdoughs with sucrose addition was 3.6% and 5.2% (flour basis), respectively (Table 5). The amount of dextran formed by *W. confusa* was higher than what could theoretically be synthesized from 10% sucrose (~ 5%), and is attributable to the presence of sucrose (2.88%) naturally existing in faba bean flour. This also explains the low amount of dextran produced by the strains in sourdoughs without added sucrose (0.9% and 0.4%, for *W. confusa* and *L. pseudomesenteroides*, respectively).

3.4 Dough mixing properties and large deformation dough rheology by Kieffer extensibility rig

Substitution of wheat with 30% faba bean flour generated a significant decrease in dough consistency and water absorption (WA) compared to 100% wheat flour (Table 6). Chemical acidification of the faba bean flour led to a slight increase in consistency and WA compared to the FWB control, but it was still significantly lower than the wheat control. Fermentation of faba bean with the selected strains compensated for the negative effect. Regardless of the strain used, doughs prepared with EPS-negative sourdoughs showed consistency and WA similar to wheat control doughs. For dextran-enriched sourdoughs, a significantly higher dough consistency and WA was observed compared to the wheat control doughs.

In the Kieffer extensibility analysis, substitution of wheat flour with faba bean flour with or without sourdough fermentation led to a significant decrease of R_{max} (maximum resistance to extension) and correspondingly a notable reduction of A_{tot} (total area) for all doughs (Table 6). Chemical acidification significantly increased the R_{max} but not A_{tot} compared to FW control doughs. Dextran production did not account for major changes in R_{max} and A_{tot} in SD doughs prepared with *L. pseudomesenteroides* and they were not significantly different from the unfermented FW control doughs. Compared to *L. pseudomesenteroides* SD doughs, a different trend was observed for *W. confusa* SD doughs. There was a significant increase of R_{max} and A_{tot} in doughs prepared with dextran-enriched *W. confusa* sourdough, which was roughly double compared to the FW control doughs and significantly higher than in the CA control doughs. *W. confusa* EPS-negative sourdough showed a significant increase in R_{max} and A_{tot} but this was slightly lower than in its dextran-enriched counterpart. The maximum extensibility (Ext) increased significantly upon replacement of wheat flour with faba bean flour (FW control dough) (Table 6). Chemically acidified and fermented faba bean flour decreased the Ext to a different degree than the wheat control.

3.5 Fundamental rheology

311 The viscoelastic properties of the doughs were evaluated using oscillatory measurements to assess the
312 microstructural changes occurring due to the substitution of wheat with faba bean flour and the influence
313 of acidification and dextran enrichment. All samples exhibited a higher elastic modulus (G') than viscous
314 modulus (G''), indicating that all the doughs had a solid, elastic-like behavior (data not shown). Generally,
315 FW control doughs had a significantly lower G' than the other doughs (Figure 2A), indicating a
316 weakening of the dough gel network with the faba bean flour substitution. However, chemical
317 acidification and incorporation of sourdoughs resulted in higher G' values than the FW control doughs.
318 The G' values of EPS-positive SD doughs and the corresponding CA control doughs were similar.
319 Irrespective of the strain used, dough with EPS-positive sourdough addition showed significantly reduced
320 G' compared to dough prepared with negative sourdough ($p<0.05$) (Figure 2A1 and A2), indicating the
321 softening effect of dextran.

322 The effect of dextran formed *in situ* and acidification on the phase angle (δ) of doughs is shown in Figure
323 2B. The changes in δ induced by substitution of wheat flour with faba bean flour were frequency
324 dependent. At medium and high frequency, wheat doughs showed a higher δ compared to FW control
325 doughs. The CA control doughs showed lower δ values than the FW control doughs in the whole
326 frequency range. In comparison to the wheat doughs, FW control doughs and CA control doughs, a
327 notable increase of δ was observed upon the inclusion of sourdough, indicating a decrease of elasticity
328 ($p<0.05$). Independently of the strain used, remarkably higher δ values were obtained in doughs prepared
329 with EPS-positive sourdoughs than in their EPS-negative counterparts.

330 *3.6 Bread quality*

331 The effect of dextran formation on bread quality is summarized in Table 7. The amount of baking loss in
332 the control WB and FWB dropped significantly upon addition of sourdough or CA control dough. The
333 greatest baking loss occurred with the control FWB. The substitution of wheat flour with faba bean flour

showed a significant decrease in loaf specific volume compared to wheat flour alone. Incorporation of CA control dough resulted in a significant decrease in specific volume compared to the control FWB. Meanwhile, the addition of EPS-positive *L. pseudomesenteroides* sourdough led to a further dramatic decrease of specific volume compared to the control FWB and CAB. EPS-negative *L. pseudomesenteroides* FSB showed a similar drop in specific volume compared to the control FWB. In contrast, adding *W. confusa* sourdoughs improved the specific volume. The highest specific volume, 21% higher than the control FWB and 8% higher than control WB, was obtained with addition of EPS-positive *W. confusa* sourdough. The inclusion of EPS-negative *W. confusa* sourdough also improved the specific volume (+12%) compared with the control FWB, to levels comparable to the control WB.

Wheat flour substitution furthermore led to a significant increase of crumb hardness ($p<0.05$) compared to the control WB (Figure 3). Incorporation of sourdough or CA control dough increased the crumb hardness for all the breads. The hardening effect was more strongly emphasized with EPS-positive *L. pseudomesenteroides* sourdough, which showed the highest crumb firmness independently of storage time. Only addition of EPS-positive *W. confusa* sourdough positively influenced the crumb structure, resulting in the softest crumb among the composite breads and comparable to the control WB. However, after 4 days of storage the crumb of the EPS-positive *W. confusa* FSB became harder than that of the control WB.

4. Discussion

Faba bean is rich in proteins and bioactive compounds but has not been extensively utilized in bakery products due to the presence of ANF and poor textural/sensory quality. Fermentation of faba bean flour with the simultaneous production of dextran is a potential option to compensate for the quality losses. The *in situ* produced dextran essentially acting as hydrocolloid, improves the technological properties of the dough and final bread product. Previously, dextran produced *in situ* affected the rheological

properties of faba bean sourdough by thickening and improving the overall elasticity of the sourdough (Xu et al., 2017). Additionally, the positive effect of faba bean sourdough on the nutritional quality of composite wheat bread has been shown (Coda et al., 2017b). In this study, the influence of faba bean sourdough containing *in situ* produced dextran on the quality of composite wheat bread is investigated by comparing the influence of dextran formation by *W. confusa* and *L. pseudomesenteroides* on the rheology and quality of wheat dough and breads containing 30% faba bean flour. Previous studies have shown that the performance of EPS-producing starters depends on the EPS yield, EPS macromolecular properties and amounts of organic acids formed (Kaditzky, Seitter, Hertel, & Vogel, 2008).

The content of dextran synthesized *in situ* by the lactic acid bacteria starters was 3.6–5.2% (flour basis) in the sourdough and consequently the final breads contained 1.1–1.6% dextran (flour basis), which was in the range (0.1–2%) of commercial hydrocolloids such as HPMC, CMC, GG and κ-CAR applied in baking (Ferrero, 2017). Dextran synthesized by *W. confusa* resulted in a more viscous sourdough compared to sourdough with *L. pseudomesenteroides* dextran. This might be due to the higher content of dextran produced by *W. confusa* but might also reflect differences in the dextrans produced, such as Mw and degree of branching (Lacaze, Wick, & Cappelle, 2007; Rühmkorf et al., 2012; Zhang et al., 2018). Furthermore, addition of dextran-enriched sourdoughs into bread doughs significantly increased the farinograph maximum consistency and WA compared to control bread doughs. Generally, addition of hydrocolloids in bread dough increases farinograph water absorption, which is related to the hydrophilic nature and water binding capacity of the hydrocolloid (Guarda et al., 2004).

The lactic acid bacteria was found to be the dominate group at the end of fermentation, which indicates a low presence of other spontaneous microbial groups (Coda et al., 2017a). Based on the sugar analysis, glucose released from sucrose was completely utilized by both strains, mainly for dextran production. In EPS-positive faba bean sourdoughs fermented with *W. confusa*, a nearly theoretical amount of fructose

was accumulated, while *L. pseudomesenteroides* due to the mannitol dehydrogenase activity consumed most of the fructose, which led to a different organic acid profile (Erten, 1998; Galle, Schwab, Arendt, & Ganzle, 2010). The concentration of lactic and acetic acid in sourdoughs plays an important role in the taste and flavor of sourdough bread. The fermentation quotient (FQ) is a useful parameter in studies of sourdough for evaluating the balance of acids produced. Acetic acid is beneficial for its preservative effect (antimicrobial compounds) and sensory contribution. However, high levels of acetic acid may result in a strong sour flavor in the bread and compromise dough stability and crumb structure.

Fundamental rheological measurements (oscillation test) and empirical measurements using a large deformation Kieffer extension test were used to evaluate the viscoelastic properties of the bread dough. The elastic component of a material is measured as the storage modulus (G'). The ratio between the viscous and elastic modulus is the tangent of the phase angle (δ). The larger the phase angle, the more viscous the material. Substitution of wheat flour with faba bean flour significantly reduced the dough elasticity (decreased G' and increased δ at low frequency). This may be attributed to the reduced wheat-gluten content and increased fiber content of faba bean flour which hinders gluten network formation. Inclusion of faba bean sourdough significantly changed the bread dough rheology, resulting in an increased elastic component (G') and more viscous dough (increased δ) compared to the FW control. However, dextran-enriched sourdoughs reduced the bread dough elasticity (decreased G' and increased δ) compared to doughs prepared with EPS-negative sourdough. The effect of acidification and presence of hydrocolloids on the elasticity of the composite bread dough is not fully understood. The weakening effect of dextran and other hydrocolloids on the elasticity of wheat dough and gluten-free dough has been reported before (Galle et al., 2012a, 2012b; Rosell et al., 2001; Wolter, Hager, Zannini, Czerny, & Arendt, 2014). Additionally, acid formation leads to unfolding of gluten proteins with an increased electronic repulsion force, which interferes with network formation and thus weakens the gluten structure (Galal,

Varrianomarston, & Johnson, 1978). Furthermore, an acidic environment activates proteolytic enzymes, which may induce depolymerization of gluten during proofing (Clarke, Schober, Dockery, O'Sullivan, & Arendt, 2004). Thus the influence of the *W. confusa* and *L. pseudomesenteroides* sourdoughs on the rheology of the composite wheat bread dough resulted from the combined effect of acidity and dextran on development of the gluten network.

Adequate extensibility is essential for appropriate dough handling and bread baking performance. R_{max}, Ext and A_{tot} (strength value) are important parameters for evaluating dough extensibility (Smewing, 1995). The observed reduction in R_{max} and A_{tot} and increase in Ext with replacement of wheat with faba bean flour indicates a more flowy dough lacking stability. The use of *L. pseudomesenteroides* sourdoughs did not influence the bread dough extension properties (R_{max} and A_{tot}), whereas *W. confusa* significantly increased both of them. In particular, bread dough containing *W. confusa* dextran showed the highest effect. The increase of those parameters promoted by hydrocolloid incorporation indicates a better dough tolerance during proofing stage (Rosell et al., 2001).

Overall, the rheological parameters most closely related to loaf specific volume and crumb firmness are the Kieffer extensibility parameters R_{max} and A_{tot}. However in this study the Ext from the Kieffer analysis and also the elastic modulus G', phase angle (δ) from oscillatory measurements, could not predict baking performance. In previous studies, R_{max} (Dobraszczyk & Salmanowicz, 2008; Kieffer, Wieser, Henderson, & Graveland, 1998) and A_{tot} (Nash et al., 2006) were positively correlated with bread volume, while Ext was not correlated with baking quality. Oscillatory measurements have proven inadequate for predicting baking performance due to the inappropriate deformation conditions (small shear deformation and high strain rate) compared to actual deformation during dough proofing and baking (large extensional deformation, lower strain rates and higher temperature) (Dobraszczyk & Morgenstern, 2003; Safari-Ardi & Phan-Thien, 1998). However, there may be additional factors beyond dextran production and

426 acidification accounting for the rheological properties of bread dough containing sourdough and bread
427 quality, as previously reported (Clarke, Schober, & Arendt, 2002; Kaditzky et al., 2008).

428 In agreement with earlier studies (Coda et al., 2017b), the substitution of wheat with faba bean flour or
429 chemically acidified dough resulted in a significant decrease in bread volume accompanied by a dramatic
430 increase in crumb hardness, leading to inferior quality of the wheat bread. Addition of sourdoughs
431 fermented by *W. confusa* compensated for the negative effect of acids and diluted gluten, improving the
432 bread volume to levels comparable to that of wheat bread. Notably, dextran containing sourdough
433 fermented by *W. confusa* showed the greatest volume improvement and crumb softness. Most likely, the
434 gluten-dextran interactions resulted in additional strength to the gas cells and hence prevented diffusion
435 and collapse of the gas cells during proofing and baking (Bárcenas & Rosell, 2005). This, combined with
436 their water binding capacity, leads to a higher loaf volume and softer crumb. Additionally, hydrocolloids
437 have a weakening effect on starch structure due to the inhibition of amylose leaching and crystallization,
438 and amylopectin retrogradation, thus modifying the water distribution and moisture retention in the bread
439 crumb (Biliaderis, Arvanitoyannis, Izydorczyk, & Prokopowich, 1997). In contrast, the incorporation of
440 sourdough containing dextran synthetised by *L. pseudomesenteroides* did not achieve the same positive
441 effect on the bread quality, which exhibited the lowest volume and hardest crumb. This was also due to
442 the higher acidity which counteracts the potentially beneficial effect of dextran. Intensive acidification
443 negatively affects loaf volume, crumb hardness and firming kinetics (Kaditzky et al., 2008). It should be
444 noted that the polymer properties (Mw and structure) of synthesized dextran also influence bread quality
445 and should also be evaluated in future work to give a complete picture.

446 In conclusion, sourdough containing dextran synthetized by *L. pseudomesenteroides* and characterized
447 by higher acidity reduced dough strength, loaf volume and crumb softness. Sourdough fermented by *W.*
448 *confusa* on the other hand, formed substantial amounts of dextran but low concentration of acids,

449 resulting in a wheat-faba bean composite bread with improved loaf volume and crumb softness. Dextran-
450 enriched faba bean sourdough could be used at a higher level (43% of the dough weight) in wheat bread
451 baking, resulting in bread with a high protein content. Furthermore, the application of dextran *in situ*
452 allows a "clean label" product which is the option for hydrocolloid utilization preferred by both industry
453 and consumers.

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587

Figure captions

Figure 1. Apparent viscosities as a function of shear rate of faba bean sourdoughs (24h) fermented with: *W. confusa* E3403 with 10% sucrose (-○-), *W. confusa* E3403 without sucrose (-■-), *L. pseudomesenteroides* DSM 20193 with 10% sucrose (-Δ-), and *L. pseudomesenteroides* DSM 20193 without sucrose (-▼-). Non-fermented faba bean sourdough (time 0) (-♦-) served as the control. The error bars represent the standard deviation (n=3). Different lowercase letters indicate significant differences ($p < 0.05$) among different types of sourdough at the same shear rate.

Figure 2. Effect of EPS on the elastic modulus (G') (A) and phase angle δ (B) of faba bean-wheat doughs with 42.5% sourdough addition fermented with *L. pseudomesenteroides* DSM 20193 (1) and *W. confusa* E3403 (2). Doughs with sourdough containing 10% sucrose (-◊-) were compared with doughs with sourdough without sucrose (-∇-), wheat doughs (-■-), faba bean-wheat doughs without sourdough addition (-●-), and doughs with chemically acidified dough (-★-) served as the control (closed symbol). The error bars represent the standard deviation (n=3).

Figure 3. Crumb hardness at different storage times (day 1 and day 4) for analyzed breads. The data is presented as a percentage based on the control FWB (100%). The error bars represent the standard deviation (n=12). Different lowercase letters indicate significant differences ($p < 0.05$) among the eight types of bread after 1 day of storage; the uppercase letters indicate significant differences ($p < 0.05$) after 4 days of storage.

Fig. 1

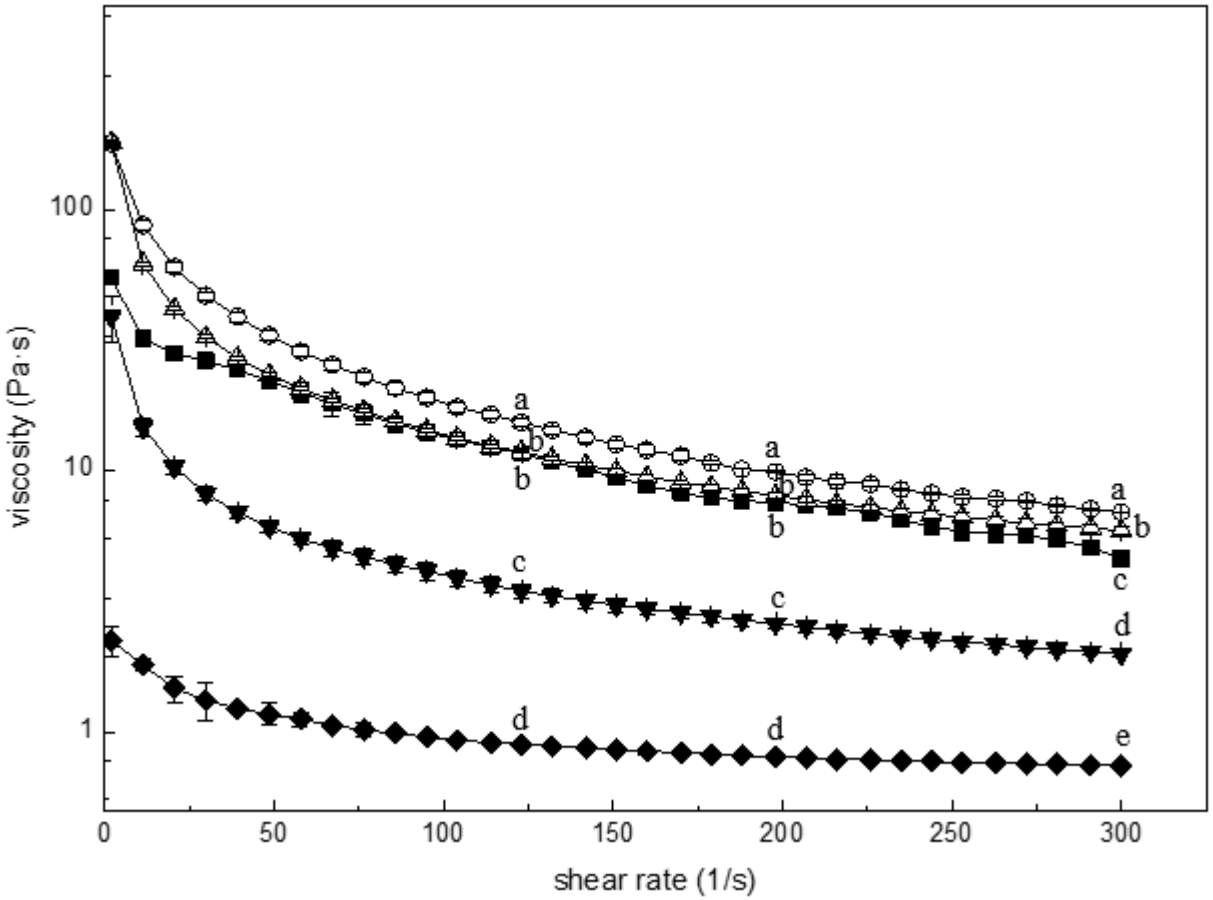


Fig. 2

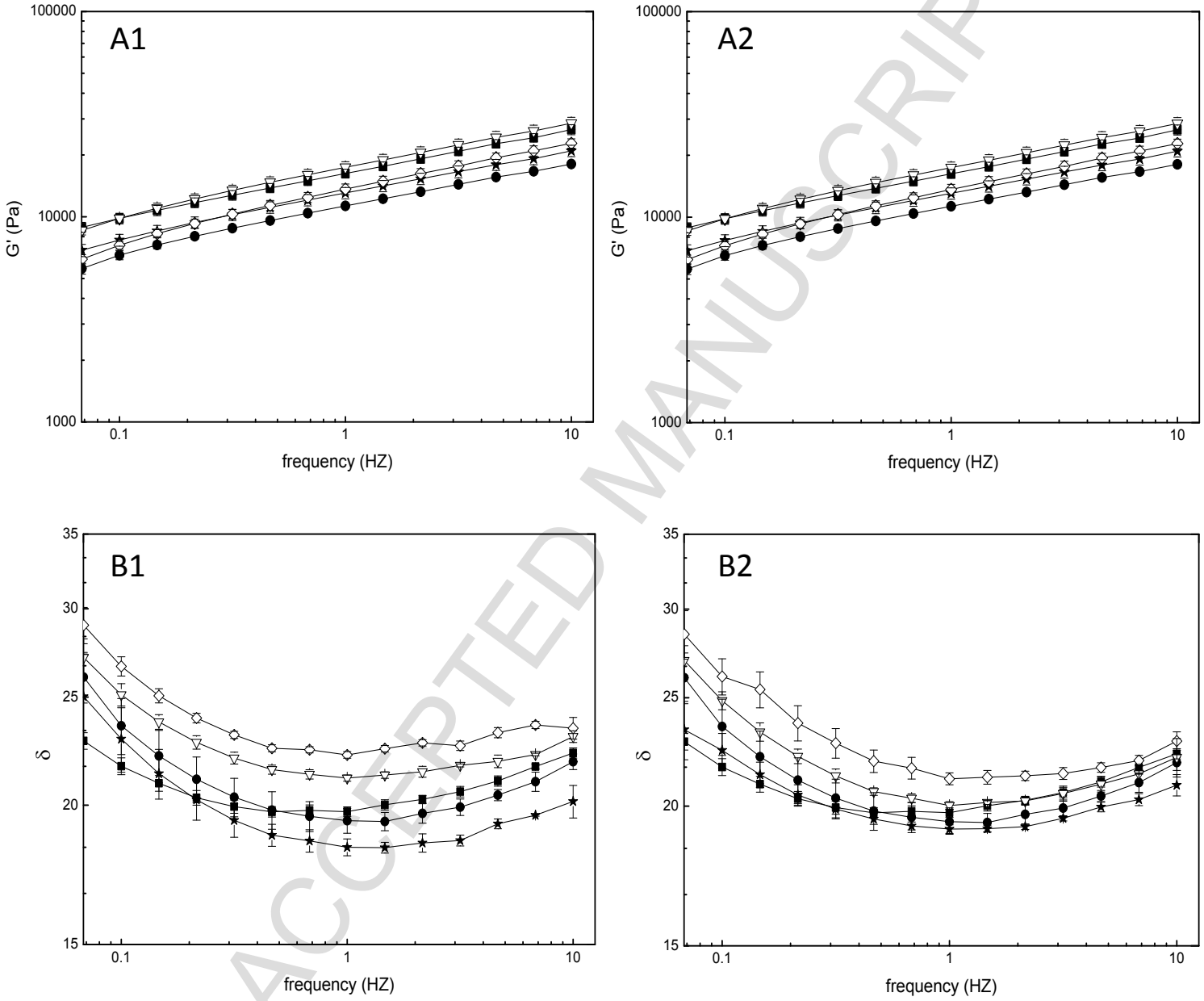
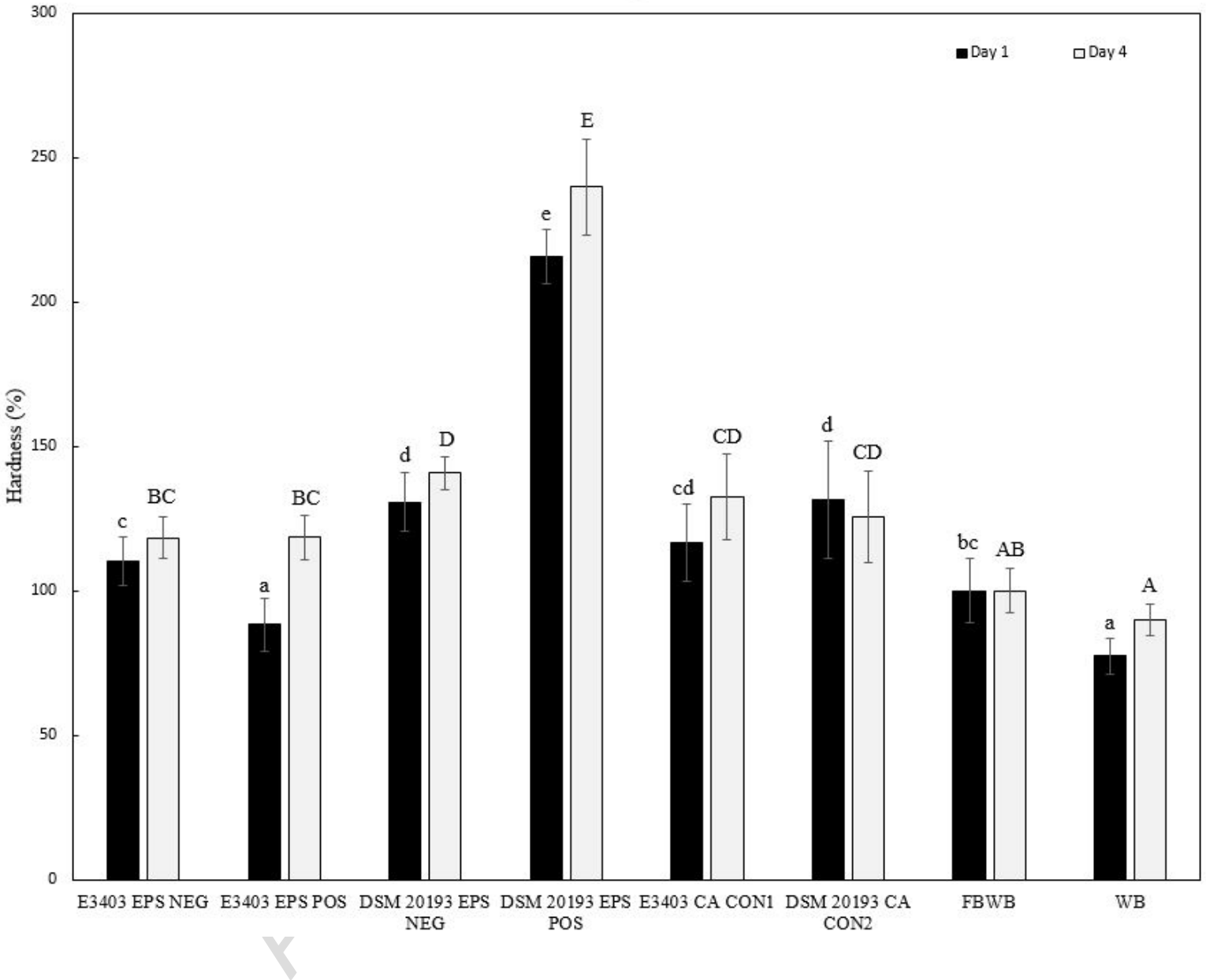


Fig. 3


Day 1
Day 4
Hardness (%)
300
250
200
150
100
50
0
c
BC
a
BC
d
D
e
E
cd
CD
d
CD
bc
AB
a
A
E3403 EPS NEG
E3403 EPS POS
DSM 20193 EPS NEG
DSM 20193 EPS POS
E3403 CA CON1
DSM 20193 CA CON2
FBWB
WB

Highlights

1. Faba bean (30%) enriched wheat breads baked with and without sourdough.
2. *W. confusa*-dextran increased sourdough viscosity and bread dough strength.
3. *W. confusa*-dextran sourdough improved textural qualities of the composite bread.
4. Excessive acidification had negative effect on bread volume and crumb structure.

Faba bean flour

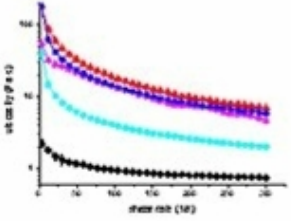
In situ
Microbial dextran

Faba bean sourdough

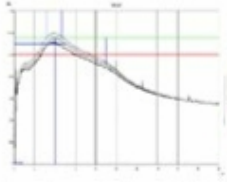
Protein enriched
wheat-faba bean bread

Increased
Volume

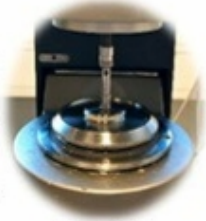
Decreased
firmness



Increased
viscosity



Farinograph:
increased water
absorption



Oscillation
rheology:
increased G'

Extensibility:
increased
dough strength

Table 1. Recipes for faba bean sourdoughs and different bread doughs.

	Wheat bread (WB)		Faba bean wheat bread (FWB)		Chemically acidified bread (CAB)				Sourdough bread (FSB)			
					E3403 CA CON1		DSM20193 CA CON2		EPS NEG SD		EPS POS SD	
	g	% f.w.	g	% f.w.	g	% f.w.	g	% f.w.	g	% f.w.	g	% f.w.
FB flour					437.4	30.0	437.4	30.0	437.4	30.0	393.7	27.0
Water					656	45.0	656	45.0	656	45.0	656	45.0
Sucrose											43.7	3.0
Acetic acid					1.4	0.1	2.7	0.2				
Lactic acid					3.4	0.2	3.9	0.3				
Breads												
CA dough /SD					1092.4	74.9	1092.4	74.9	1092.4	74.9	1092.4	74.9
FB flour			437.4	30.0								
Wheat flour	1458	100.0	1020.6	70.0	1020.6	70.0	1020.6	70.0	1020.6	70.0	1020.6	70.0
Water	918.5	63.0	918.5	63.0	263.5	18.1	263.5	18.1	263.5	18.1	263.5	18.1
Yeast	72.9	5.0	72.9	5.0	72.9	5.0	72.9	5.0	72.9	5.0	72.9	5.0
Sugar	29.2	2.0	29.2	2.0	29.2	2.0	29.2	2.0	29.2	2.0	29.2	2.0
Salt	21.9	1.5	21.9	1.5	21.9	1.5	21.9	1.5	21.9	1.5	21.9	1.5
Fat	72.9	5.0	72.9	5.0	72.9	5.0	72.9	5.0	72.9	5.0	72.9	5.0
Flour Sum	1458	100.0	1458	100.0	1458	100.0	1458	100.0	1458	100.0	1458	100.0

EPS NEG SD = EPS-negative sourdough. EPS POS SD = EPS-positive sourdough. f.w. = flour weight.

Table 2. Amount of energy-containing nutrients in bread dough (faba bean wheat control dough and EPS-negative sourdough dough) and their energy contents (%).

	Ingredient Amount (g/100g dough)	Carbohydrates		Fiber		Protein		Fat	
		Con. (%)	Amount (g/100g dough)	Con.(%)	Amount (g/100g dough)	Con. (%)	Amount (g/100g dough)	Con. (%)	Amount (g/100g dough)
Wheat flour	39.7	65.0	25.81	5.3	2.10	14.0	5.56	1.7	0.67
Faba bean flour	17.0	37.5	6.38	16.0	2.72	30.0	5.10	1.5	0.26
Fresh yeast	2.8	1.1	0.03	6.9	0.19	13.1	0.37	0.4	0.01
Sugar	1.1	100.0	1.10	0.0	0.00	0.0	0.00	0.0	0.00
Fat	2.8	0.0	0.00	0.0	0.00	0.0	0.00	80.0	2.24
Sum			33.31		5.02		11.02		3.18
Energy content (kcal/g)			4.00		2.00		4.00		9.00
Energy content (kcal/100g dough)			133.24		10.03		44.10		28.63
Energy content (%)			61.68		4.65		20.42		13.25

Con. = concentration.

Table 3. Amount of energy-containing nutrients in the bread dough (EPS-positive sourdough dough) and their energy contents (%).

	Ingredient Amount (g/100g dough)	Carbohydrates		Fiber		Protein		Fat	
		Con. (%)	Amount (g/100g dough)	Con.(%)	Amount (g/100g dough)	Con. (%)	Amount (g/100g dough)	Con. (%)	Amount (g/100g dough)
Wheat flour	40.4	65.0	26.26	5.3	2.14	14.0	5.66	1.7	0.69
Faba bean flour	15.5	37.5	5.81	16.0	2.48	30.0	4.65	1.5	0.23
Fresh yeast	2.9	1.1	0.03	6.9	0.20	13.1	0.38	0.4	0.01
Sugar	1.2	100.0	1.20	0.0	0.00	0.0	0.00	0.0	0.00
Fat	2.9	0.0	0.00	0.0	0.00	0.0	0.00	80.0	2.32
Sum			33.30		4.82		10.69		3.25
Energy content (kcal/g)			4.00		2.00		4.00		9.00
Energy content (kcal/100g dough)			133.22		9.64		42.74		29.26
Energy content (%)			62.00		4.49		19.89		13.62

Table 4. Number of total bacteria and lactic acid bacteria (log cfu/g), acidity (pH and TTA) of faba bean sourdoughs before and after fermentation for 24 h, and bread crumb.

	SD 0h				SD 24h				Bread crumb	
	pH	TTA (ml)	Lactic acid bacteria	Total bacteria count	pH	TTA (ml)	Lactic acid bacteria	Total bacteria count	pH	TTA (ml)
E3403 EPS NEG	6.5 ± 0.0^{a}	4.2 ± 0.1^{a}	6.1 ± 0.1^{a}	6.3 ± 0.1^{a}	5.6 ± 0.0^{a}	8.8 ± 0.3^{a}	9.5 ± 0.1^{a}	9.7 ± 0.1^{a}	5.3 ± 0.0^{c}	6.3 ± 0.1^{c}
E3403 EPS POS	6.4 ± 0.0^{a}	3.8 ± 0.1^{bc}	6.1 ± 0.1^{a}	6.2 ± 0.2^{a}	5.1 ± 0.0^{b}	10.0 ± 0.3^{b}	9.5 ± 0.1^{a}	9.6 ± 0.1^{a}	5.0 ± 0.0^{d}	7.0 ± 0.2^{c}
DSM 20193 EPS NEG	6.5 ± 0.0^{a}	4.1 ± 0.0^{ab}	6.1 ± 0.2^{a}	6.3 ± 0.1^{a}	4.7 ± 0.1^{c}	16.4 ± 0.4^{c}	9.9 ± 0.1^{b}	9.9 ± 0.0^{b}	4.7 ± 0.0^{e}	9.7 ± 0.6^{d}
DSM 20193 EPS POS	6.5 ± 0.0^{a}	3.7 ± 0.2^{c}	5.9 ± 0.0^{a}	6.2 ± 0.1^{a}	4.6 ± 0.0^{c}	18.5 ± 0.2^{d}	9.8 ± 0.0^{b}	9.8 ± 0.1^{b}	4.6 ± 0.0^{e}	10.2 ± 0.6^{d}
FWB									6.0 ± 0.0^{a}	4.2 ± 0.1^{b}
WB									5.7 ± 0.0^{b}	2.4 ± 0.1^{a}

Different letters in the same column indicate statistical significance ($p<0.05$).

Table 5. Amount of sucrose, fructose, glucose and dextran (% of flour weight) in various sourdoughs and organic acid formation after 24 h fermentation.

Type of SD	Sucrose (%)	Glucose (%)	Fructose (%)	Lactic acid (g / kg SD)	Acetic acid (g / kg SD)	FQ	Dextran (%)
E3403 EPS NEG	nd	nd	0.82 ± 0.01c	3.2 ± 0.3a	1.4 ± 0.0a	1.5 ± 0.0b	0.86 ± 0.02c
E3403 EPS POS	nd	nd	4.59 ± 0.03a	3.6 ± 0.2a	1.3 ± 0.1a	1.8 ± 0.0b	5.19 ± 0.01a
DSM 20193 EPS NEG	nd	nd	0.08 ± 0.01d	4.7 ± 0.3b	1.2 ± 0.3a	2.6 ± 0.4a	0.44 ± 0.02d
DSM 20193 EPS POS	nd	nd	1.47 ± 0.03b	3.5 ± 0.2a	2.8 ± 0.2b	0.9 ± 0.0c	3.63 ± 0.08b

Different letters in the same column indicate statistical significance ($p<0.05$). FQ = fermentation quotient. nd = not detected.

Table 6. Parameters from the Brabender farinograph with water absorption corrected to 500 BU and Kieffer extensigraph for wheat control dough, faba-wheat control dough, chemically acidified control dough and sourdough dough.

	Wheat dough	FW dough	CA dough		SD dough			
			E3403 CA CON1	DSM20193 CA CON2	E3403 EPS NEG	E3403 EPS POS	DSM 20193 EPS NEG	DSM 20193 EPS POS
Consistency	503 ± 1c	400 ± 1e	451 ± 2d	452 ± 2d	497 ± 3c	578 ± 2b	504 ± 2c	591 ± 1a
WA (%)	70.9 ± 0.0b	68.3 ± 0.1d	69.6 ± 0.1c	69.6 ± 0.1c	70.7 ± 0.0b	72.8 ± 0.2a	70.9 ± 0.1b	73.1 ± 0.1a
R_{max} (g)	30.5 ± 2.0a	7.7 ± 0.7d	10.7 ± 0.8c	10.9 ± 0.8c	13.5 ± 1.1b	15.0 ± 1.1b	7.4 ± 0.4d	7.9 ± 0.3d
Ext (cm)	4.7 ± 0.1bc	5.7 ± 0.5a	3.4 ± 0.3de	3.0 ± 0.5e	4.4 ± 0.6cd	3.2 ± 0.4e	2.6 ± 0.4e	4.5 ± 0.6cd
A_{tot} (mm^2)	611.5 ± 4.3a	250.9 ± 2.2c	266.5 ± 2.8c	254.8 ± 4.0 c	357.0 ± 4.2 b	377.6 ± 4.5b	167.6 ± 2.1d	208.7 ± 2.2cd

Different letters in the same row indicate statistical significance ($p<0.05$). WA = farinograph water absorption. R_{max} = maximum resistance to extension (g). Ext = extensibility (cm). A_{tot} = total area under the curve.

Table 7. Baking characteristics of breads.

	WB	FWB	CAB		FSB			
			E3403 CA CON1	DSM20193 CA CON2	E3403 EPS NEG	E3403 EPS POS	DSM 20193 EPS NEG	DSM 20193 EPS POS
Baking loss (%)	11.8 ± 0.2ab	12.0 ± 0.6a	10.0 ± 0.5d	10.0 ± 0.6d	11.0 ± 0.4c	11.8 ± 0.5ab	11.4 ± 0.3bc	10.3 ± 0.3d
Sp. volume (ml/g)	3.8 ± 0.1b	3.4 ± 0.0c	3.2 ± 0.1d	3.0 ± 0.1de	3.8 ± 0.1b	4.1 ± 0.1a	3.4 ± 0.1c	2.9 ± 0.1e

Different letters in the same row indicate statistical significance ($p<0.05$). Sp. volume = specific volume.