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In situ production of vitamin B12 and dextran in soya flour and rice bran: A tool to improve flavour and texture of B12-fortified bread

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ABSTRACT

This study aimed to develop a fermentation process that allows the concomitant production of dextran (a texture-enhancing agent) and vitamin B12 (*in situ* fortification) for bread applications. Mixed fermentation of soya flour or rice bran using *Propionibacterium freudenreichii* DSM 20271 and *Weissella confusa* A16 with added sucrose resulted in substantial quantities of dextran (5.6–5.8% dry matter) and B12 (7.9–8.9 μg/100 g fresh weight), together with antifungal metabolites (e.g. acetic and propionic acids). In addition to an extended mould-free shelf life, the bread containing 50% (dough weight) fermented soya flour or rice bran not only contained adequate levels of B12 but also exhibited improved texture and sensory quality compared to the control, such as a higher loaf volume, a softer crumb, and a more cohesive and moister mouthfeel. The mixed fermentation reduced the beany or cooked-rice flavour but increased sour and cheesy flavours and aftertaste. Dextran produced during the fermentation process exhibited a masking effect on the beany and sour notes and aftertaste, consistently with reduced levels of green or grassy volatiles (e.g. hexanal, heptanal, (*E*,*E*)-2,4-decadienal, and 2-pentylfuran). Overall, the mixed fermentation method using *P. freudenreichii* DSM 20271 and *W. confusa* A16 showed potential for B12 fortification of bread products with high sensory quality.

1. Introduction

Vitamin B12 (cobalamin, B12) is essential for humans as a cofactor (Kräutler, 2012). B12 biosynthesis is limited to certain bacteria and archaea (Roth, Lawrence, & Bobik, 1996) and its dietary sources are almost exclusively of animal origin (Combs & McClung, 2017). Therefore, subclinical B12 deficiency is likely prevalent and has been observed in vegans and vegetarians and in the elderly due to malabsorption (Butola et al., 2020; Green et al., 2017). *In situ* fortification of plant-based products with B12 via microbial fermentation is an economically beneficial and natural technique that has yet to be exploited on a large scale to provide adequate B12 intake for populations at risk of B12 deficiency.

Propinibacterium freudenreichii, a food-grade microorganism, has been used for *in situ* fortification of the human active form of B12 in plant-based foods such as bread (Edelmann, Chamlagain, Santin, Kariluoto, & Piironen, 2016) and pasta (Chamlagain et al., 2021). In non-sterile plant materials, fermentation with *P. freudenreichii* monoculture is unable to inhibit the growth of potentially endogenous pathogens. Therefore, mixed fermentation using *P. freudenreichii* and lactic acid bacteria (LAB) has been introduced to improve the microbiological safety of the fermented plant material (Xie et al., 2019, 2021). An additional benefit of mixed fermentation using *P. freudenreichii* in tandem with LAB is enhanced levels of B12 production, especially in brans and legume flours that are known for their salutary effects on human health (Xie et al., 2021). Of note, rice bran is one of the most abundant by-products produced during the rice-milling process and is rich in numerous bioactive compounds (such as lipids, minerals, vitamins, dietary fibres, phytonutrients, and antioxidants), many of which have been widely studied and shown to exhibit beneficial effects *in vitro* and *in vivo* (Sapwarobol, Saphyakhajorn, & Astina, 2021). Similarly, soya is nutrient-dense and contains all the essential amino acids necessary for human nutrition (Dukariya, Shah, Singh, & Kumar, 2020). Using soya flour as a wheat alternative in bread making contributes to an enhanced amino-acid profile, a higher protein content, and environmental sustainability (Boukid, Zannini, Carini, & Vittadini, 2019).

However, substituting wheat flour with soya flour or rice bran at

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Abbreviations

CFU	colony-forming unit
CS	control sourdough
CSB	control sourdough bread
DS	dextran-enriched sourdough
DSB	dextran-enriched sourdough bread
dw	dough weight
fw	flour weight
GEM	general edible medium
LAB	lactic acid bacteria
PL	co-culture of *P. freudenreichii* DSM 20271 and *L. brevis* ATCC 14869
PW	co-culture of *P. freudenreichii* DSM 20271 and *W. confusa* A16
TPA	texture profile analysis
TTA	total titratable acidity
HPAEC-PAD	high-performance anion-exchange chromatography with pulsed amperometric detection
HS-SPME-GC-MS	head-space solid-phase micro-extraction GC-MS
UHPLC	ultra-high performance liquid chromatography

higher levels (e.g. >15%) is hampered by the detrimental effects on textural and sensory properties compared to regular wheat bread (Bultum, Emire, & Wolde, 2020; Haque, Hossain, Zim, Aziz, & Hoque, 2020). Furthermore, soya flour and rice bran have high lipid contents and are susceptible to oxidative reactions, leading to the development of beany or rancid off-flavours. To improve the texture and sensory quality of composite breads, dextrans may be a natural replacement of commercial hydrocolloids (Wang, Maina, Coda, & Katina, 2021). Dextrans are homopolysaccharides of D-glucose consisting predominately (>50%) of α-1,6-glucopyranosidic backbone linkages and are produced extracellularly by LAB using sucrose as the substrate (Monsan et al., 2001). We recently showed that dextrans produced *in situ* by *Weissella* species can function as masking agents for off-flavours and can suppress the bitter taste and aftertaste in wholegrain sorghum bread (Wang et al., 2020).

In the present study, we hypothesized that mixed fermentation using *P. freudenreichii* and LAB may conflate the benefits of microbially produced B12 and dextrans to produce novel nutritionally fortified bread with palatable sensory profiles. Therefore, we aimed to determine the *in situ* production of B12 by *P. freudenreichii* DSM 20271 during mixed-culture fermentations with *Weissella confusa* A16 (dextran producer) or *L. brevis* ATCC 14869 (dextran-negative control) of soya flour and rice bran. We then investigated the flavour and texture perception of the breads containing 50% (dough weight, dw) of the fermented raw materials. Finally, the volatile compounds of the breads identified by headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) were associated with sensory descriptive data obtained using a trained panel.

2. Materials and methods

2.1. Materials

Commercial wheat flour (RAISIO Oyj, Finland; protein 12%, fat 2.1%, fibre 4%, moisture 13.6%), soya flour (RISENTA AB, Finland; protein 38%, fat 20%, carbohydrate 18%, fibre 13%, cobalt 68 ng/g), rice bran (NOW Real Food®, USA; protein 13%, fat 20%, fibre 20%, cobalt 166 ng/g), fresh baker's yeast (Suomen Hiiva Oy, Finland), sucrose (DanSukker, Finland), salt (Meira Oy, Finland), and fat (Bunge Finland Oy, Finland) were used for bread making.

2.2. Mixed-culture fermentations of rice bran or soya flour

Three types of B12-fortified sourdough were prepared from rice bran or soya flour (Fig. 1): dextran-enriched sourdough fermented by a mixed culture of *P. freudenreichii* DSM 20271/*W. confusa* A16 (DS-*PW*, high in dextran); control sourdough fermented by a mixed culture of *P. freudenreichii* DSM 20271/*W. confusa* A16 (CS-*PW*, low in dextran); and control sourdough fermented by a mixed culture of *P. freudenreichii* DSM 20271/*L. brevis* ATCC 14869 (CS-*PL,* without dextran) (refer to Supplementary Material Table S1 for formulations). Dextran-enriched sourdough was made by replacing 10% (flour weight, fw) of the rice bran or soya flour with sucrose as the substrate for dextran synthesis. Control sourdough was made without sucrose supplementation. Rice bran or soya flour and distilled water was mixed at a ratio of 1:4 and strains were subsequently inoculated as described (Supplementary Material Method 1). Fermentations were performed in triplicate at 25 °C for 24 h under mild aerobic conditions with constant shaking at 150 rpm. The pH and total titratable acidity (TTA, ml 0.1 mol/L NaOH/10 g of sourdough) of the sourdoughs were determined before and after 24 h of fermentation using a Mettler Toledo 340 pH meter (Leicester, UK) and Mettler DL35 Manual Titrator, respectively (Wang et al., 2018).

2.3. Determination of cell counts, dextran, sugars, and acids in sourdoughs

The cell counts of LAB, total mesophilic aerobic bacteria, *Enterobacteriaceae*, propionibacteria (PAB), and *B. cereus* were determined at 0 h and after 24 h of fermentation (details in Supplementary Material Method 2). The dextran content in the fermented samples was determined using an enzyme-assisted method according to Katina et al. (2009). High-performance anion-exchange chromatography with pulse amperometric detection system (HPAEC-PAD) was used for quantification of monosaccharides and disaccharides (Xu et al., 2017). Analysis of lactic acid, acetic acid, and propionic acid was performed using high-performance liquid chromatography (HPLC) (Wang et al., 2019, 2020). Detailed descriptions of the qualification methods are provided in Supplementary Material Method 3.

2.4. Bread making

There were nine types of bread prepared in this study (Fig. 1): Type 1, wheat control bread; Type 2, rice bran control (i.e. wheat bread with 18.5% fw native rice bran); Type 3, soya control (i.e. wheat bread with 18.5% fw native soya flour); Types 4 and 5, rice bran DSB-*PW* and soya DSB-*PW* (i.e. wheat bread with 50% dw dextran-enriched rice bran or soya sourdough fermented by a mixed culture of *P. freudenreichii* DSM 20271/*W. confusa* A16); Types 6 and 7, rice bran CSB-*PW* & soya CSB-*PW* (i.e. wheat bread with 50% dw control rice bran or soya sourdough fermented by a mixed culture of *P. freudenreichii* DSM 20271/*W. confusa* A16); Types 8 and 9, rice bran CSB-*PL* & soya CSB-*PL* (i.e. wheat bread with 50% dw control rice bran or soya sourdough fermented by a mixed culture of *P. freudenreichii* DSM 20271/*L. brevis* ATCC 14869) (for formulation see Supplementary Material Table S1). The optimal water absorption of wheat flour (64% fw) was determined by the AACC method 54–21 (AACC International, 2000) using a Brabender Farinograph (Brabender GmbH & Co.KG, Duisburg, Germany) with a 300-g mixing bowl. The water content in all composite breads was the same (74% fw), which was determined by preliminary mixing trials in a Diosna kneader (Osnabrück, Germany) and a subjective assessment. Three independent baking batches were performed for each formulation according to Wang et al. (2020).

To prepare samples for B12 analysis, breads (two from each trial) were cut into 25-mm (thickness) slices after 1 h of baking. Two slices of each bread were selected and the crusts were removed. The bread slices were cut into small pieces (approximately 25 g) and spread as a thin layer in aluminium-foil trays with covers before freezing at −70 °C.

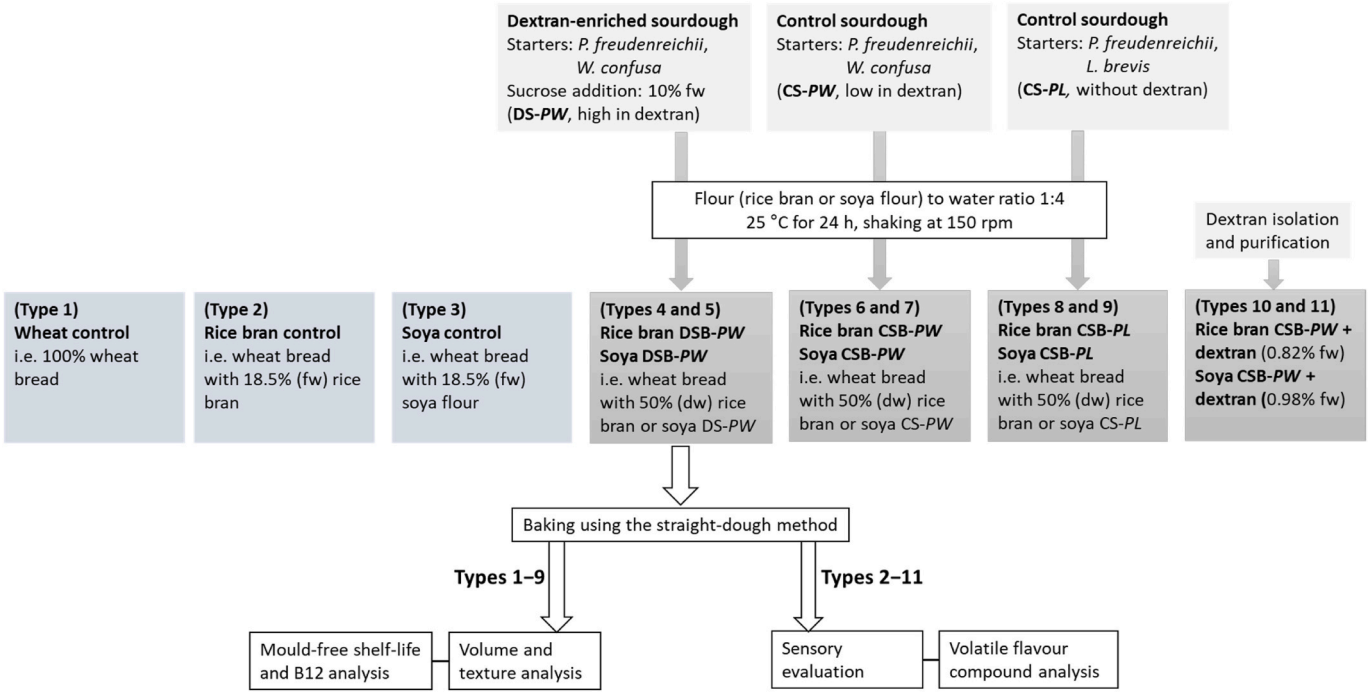

Fig. 1. Flowchart of sourdough preparation and types of bread made using sourdoughs or unfermented flours in this study.

After 48 h of deep freezing, the samples were freeze-dried using a Gamma 2–20 apparatus (Christ, Osterode a.H., Germany). The samples were weighed and milled using a high-speed blender (Oster, USA) for 60 s until completely homogeneous. The lyophilized samples were packed under vacuum (Boss Verpackungsmaschinen GmbH & Co. KG, Germany) into polyamide-polyethylene pouches and stored at −20 °C (protected from light) prior to B12 analysis.

2.5. Instrumental analysis of bread parameters and evaluation of mould-free shelf life

A Texture Profile Analysis (TPA) of bread crumbs was performed after 1 and 4 d of storage using a texture analyser TA-XT2i (Stable Micro Systems Ltd., Surrey, UK) with a compression plate, a 2-kg load cell, and a 36-mm cylinder probe (P/36R) (Wang et al., 2018). The loaf-specific volume (mL/g) was measured in triplicate by 3D digitization of the bread using a laser-based scanner (Volscan Profiler 300, Stable Micro Systems, UK). Weight loss during baking was calculated as (%bake loss = (weight of dough – weight of bread)*100/weight of dough). The acidity (pH and TTA) of the breads was determined according to Wang et al. (2018). For antifungal observations, two bread loaves and six bread slices (2.5-cm thickness with crust) from each bread formulation were packed in polyethylene bags and stored at ambient temperature (22 ± 2 °C) until the breads or slices became mouldy. The samples were visually inspected daily for the appearance of mould colonies. Mould growth was expressed as the percentage of spoiled surface to the total surface of the entire loaf or slice.

2.6. Determination of vitamin B12 content in sourdoughs and breads

Vitamin B12 was determined as cyanocobalamin using an ultra-high performance liquid chromatography (UHPLC) method as described earlier (Chamlagain, Edelmann, Kariluoto, Ollilainen, & Piironen, 2015) with minor modifications. Briefly, the defrosted sourdough samples (ca. 3 g) and freeze-dried bread samples (ca. 0.5 g) were placed in a boiling water bath for 30 min after mixing with 15 ml of extraction buffer (20.7 mmol/L acetic acid and 8.3 mmol/L sodium hydroxide, pH 4.5) and 100 μl of sodium cyanide (1% w/v in water). The solutions were allowed to cool on ice to approximately 37 °C, after which 300 μl of α-amylase (50 mg/ml; St Louis, MO, USA) was added. The samples were incubated in a water bath (30 min, 37 °C) to hydrolyse the starch before centrifugation (6900 × g, 10 min). The pellets were suspended in 5 ml of extraction buffer and the mixtures were centrifuged again. The supernatants were combined and the final volume was adjusted to 25 ml with extraction buffer. Ten mL of the extracts were purified using an immunoaffinity column (Easi-Extract; R-Biopharma; Glasgow, Scotland) and filtered (0.2 μm, Pall, USA) into UPLC vials. A Waters UHPLC system (Milford, MA, USA) equipped with a photodiode array detector (at 361 nm) and an Acquity HSS T3 C18 column (2.1 × 100 mm, 1.8 μm) was used for analysis. The mobile phase was a gradient flow of acetonitrile and Milli-Q water with 0.025% trifluoroacetic acid (0.32 ml/min). An external calibration curve for quantitation was prepared by injecting six cyanocobalamin standards (0.015–0.75 ng/μL) in duplicate.

2.7. Descriptive sensory analysis

Thirteen volunteer study participants (six females and seven males aged from 20 to 50 years) were recruited from the staff and students of the Department of Food and Nutrition at the University of Helsinki and trained as panellists. Ethical principles of sensory study at the department were assessed and approved by the University of Helsinki Ethical Review Board in the Humanities and Social and Behavioural Sciences (15/2020).

Breads containing untreated or fermented soya flour or rice bran as described in Section 2.4 (Types 2–9) were included in the sensory evaluation. Two additional breads (Types 10 and 11, Fig. 1) were included (i.e. purified dextran-supplemented soya CSB-*PW* and rice bran CSB-*PW*). Purified dextran (purity 80.5%) was added to obtain a comparable dextran content as in the respective DSB-*PW* (i.e. 0.82% (fw) in rice bran CSB-*PW* and 0.98% (fw) in soya CSB-*PW*). The food-grade dextran was synthesized by *W. confusa* A16 in GEM medium, isolated, and used in bread making according to instructions in Wang et al. (2020). The breads were baked one day before volunteer training or evaluation and were stored in polyethylene bags at room temperature. On the day of evaluation, bread samples were cut into 2.5-cm thick slices (without crust), served in covered square plastic containers to minimize

moisture loss, and coded with 3-digit random numbers. Detailed procedures for panel training and sensory evaluation are reported in Table 1 and Supplementary Material Method 4.

2.8. Volatile compound analysis by HS-SPME-GC-MS

Volatile flavour compounds of bread samples were analysed using a HS-SPME-GC-MS method, as described previously (Damerau, Kamlang-Ek, Moisio, Lampi, & Piironen, 2014; Yang, Piironen, & Lampi, 2019). The bread samples (two from each recipe) baked for sensory evaluation (Section 2.7) after 1 h of cooling were cut into 25-mm slices. Four slices were combined with the crust removed and ground with a hand blender (Bamix®, Switzerland). Approximately 1 g of samples were weighed into 20-mL headspace crimp vials sealed with magnetic screw caps, followed by a 20-min incubation period at 60 °C. Volatile compound extraction was performed at 60 °C for 30 min using a divinylbenzenecarboxen-polydimethylsiloxane (DVB/CAR/PDMS) fibre (50/30 μm). SPME was coupled to a GC (HP 6890 series, Agilent Technologies Inc., Wilmington, DE, USA) with a MS detector (Agilent 5973 Network, Agilent Technologies Inc., Wilmington, DE, USA). The GC was equipped with a SPB-624 capillary column (30 m × 0.25 mm i.d. film thickness 1.4 μm) where the volatiles were separated. Total ion chromatograms (collected in the range of 40–300 m/z) in MS were used for identification and quantification of the volatile compounds by matching against the Wiley 7N databases (Wiley Registry™ of Mass Spectral Data, 7th Edition, USA) and by comparison with the standards. The results were expressed as peak areas (counts × s × 10^6) of the triplicate samples.

Table 1
Bread descriptive sensory analysis lexicon and reference samples used in sensory evaluation.

Attribute	Description	Reference sample (scale)	Scale anchors (0, 10)
Smell			
Sour smell	Aroma associated with fermented sourdough	Wheat bread + LA/AA[a] (10)	Not at all, Very strong
Beany smell	Aroma associated with green beans or pea pods	100% soya bread (10)	Not at all, Very strong
Cooked rice smell	Aroma associated with cooked rice	100% rice bran bread (10)	Not at all, Very strong
Cheesy smell	Aroma elicited by propionic acid in Swiss-type cheeses, nutty	Wheat bread + Emmental cheese (10)	Not at all, Very strong
Overall smell intensity	Aroma overall intensity	No reference provided	Not at all, Very strong
Taste			
Cheesy	Taste elicited by propionic acid in Swiss-type cheeses, nutty	Wheat bread + Emmental cheese (10)	Not at all, Very strong
Beany	Taste associated with green beans or pea pods	100% soya bread (10)	Not at all, Very strong
Cooked rice	Aroma associated with cooked rice	100% rice bran bread (10)	Not at all, Very strong
Sour	Basic taste associated with acids	Wheat bread + LA/AA (10)	Not at all, Very strong
Bitter	Basic taste associated with caffeine and other bitter compounds	Wheat bread + caffeine (10)	Not at all, Very strong
Aftertaste	The intensity of any taste left in mouth 1 min after swallowing the bread	No reference provided	Not at all, Very strong
Mouthfeel/ texture			
Hardness	The force required to bite through sample with front teeth	Wheat bread (0)	Very soft, Very hard
Moistness	Assess the level of moisture	Wheat bread (10)	Very dry, Very moist
Cohesiveness	Assess the ability to form a ball after melting in the mouth	Wheat bread (10)	Very crumbly, Very dough-like

[a] LA, lactic acid; AA, Acetic acid.

2.9. Statistical analysis

Data collected from the chemical measurements and sensory evaluation were analysed using one-way analysis of variance (ANOVA, descriptive statistics) and univariate analysis with Turkey's post hoc test ($p < 0.05$) using the IBM SPSS Statistics 27 programme (SPSS, Inc., Chicago, IL, USA). A three-way ANOVA was performed to evaluate the significant levels of the main and interaction effects (sample, panellist, session, panellist * sample, panellist * session, and sample * session).

3. Results

3.1. Acidification and microbial growth

Rice bran sourdoughs had initial pH and TTA values of 6.8 and 0.8 mL, respectively. Soya sourdoughs had a pH of 6.3 and a TTA of 3.0 mL. After 24 h of fermentation at 25 °C, the pH decreased to 5.6–6.1 and TTA increased to 4.7–9.5 mL (Table 2). The highest acidity was observed in the rice bran control sourdough fermented by the *P. freudenreichii/ L. brevis* mixed culture (CS-*PL*), whereas the lowest acidity was obtained in soya CS-*PL*. Independent of the raw materials used, the dextran-enriched sourdoughs fermented by *P. freudenreichii/W. confusa* mixed culture with sucrose addition (DS-*PW*) showed significantly lower pH and correspondingly higher TTA (6.6–6.7 mL) than the respective control sourdoughs fermented with the same strains without sucrose addition (CS-*PW*) (TTA 5.9–6.0 mL). A similar trend was found in sourdough breads; their acidity values were significantly higher than those of the control breads.

The initial cell density of presumptive LAB and total mesophilic bacteria in all samples was similar and increased by 3.2–3.6 log cycles in soya and rice bran DS-*PW* and CS-*PW* after 24 h of fermentation, while CS-*PL* showed an increase of approximately 2.0 log cycles (Table 2). The increase in the total cell count of *Enterobacteriaceae* at the end of fermentation was significantly higher in soya and rice bran CS-*PL* than that in DS-*PW* and CS-*PW*. *B. cereus* was found at the beginning of fermentation at a cell density varying from 1.3 to 2.4 log cfu/g. After fermentation, *B. cereus* was detected in the cell density range of 0.7–2.5 log cfu/g.

3.2. Free sugars, dextran, and organic acids in sourdoughs

The native, untreated soya flour and rice bran contained 3.3 ± 0.1% and 5.9 ± 0.3% (dry matter) sucrose, respectively (data not shown). The flour endogenous sucrose was almost unused in soya and rice bran CS-*PL* during fermentation (Table 3). In contrast, no sucrose was detected in soya and rice bran CS-*PW* after fermentation, where 1.4% and 2.9% fructose accumulated and 1.3% and 2.2% dextran were synthesized, respectively. In soya and rice bran DS-*PW*, the flour intrinsic (3–6%) and supplemented (10%) sucrose was completely consumed during fermentation, leading to a significant amount of fructose release (6.6% and 7.8%, respectively) and dextran production (5.6% and 5.8%, respectively).

Concentrations of lactic acid in sourdoughs ranged from 0.73% dry matter (soya CS-*PL*) to 0.9% (rice bran CS-*PL*) (Table 3). The concentration of lactic acid was significantly higher in soya and rice bran DS-*PW* compared to their CS-*PW* counterparts. The concentrations of acetic acid were comparable (0.03–0.04%) in the sourdoughs except soya CS-*PW* (0.12%) and soya DS-*PW* (0.14%). The highest increase in propionic acid levels was found in soya DS-*PW* (0.29%), while the smallest increase was in soya CS-*PL* (0.1%).

3.3. Vitamin B12 in sourdoughs and breads

Among different types of sourdough investigated in this study, the

Table 2
Microbial growth of sourdoughs before and after fermentation (0 h and 24 h, respectively) and acidity (pH and TTA) of sourdoughs and bread crumbs.

	Cell count 0 h (log cfu/g)					Cell count 24 h (log cfu/g)					Sourdough acidity (24 h)		Bread crumb acidity	
	LAB	Total bacteria count	Enterobacteriaceae	PAB	*Bacillus cereus*[a]	LAB	Total bacteria count	Enterobacteriaceae	PAB	*Bacillus cereus*	pH	TTA (mL 0.1 mol/L NaOH)	pH	TTA (mL 0.1 mol/L NaOH)
Wheat control													5.71 ± 0.02 ^{cd}	3.23 ± 0.03 ^{a}
Rice bran control													6.17 ± 0.01 ^{e}	4.10 ± 0.11 ^{b}
Soya control													6.08 ± 0.02 ^{e}	4.32 ± 0.01 ^{bc}
Rice bran CS-*PL*[b]	6.1 ± 0.1 ^{a}	6.2 ± 0.1 ^{b}	1.1 ± 0.1 ^{a}	8.6 ± 0.1 ^{ab}	2.4 ± 0.1 ^{a}	8.0 ± 0.1 ^{a}	8.2 ± 0.1 ^{a}	4.7 ± 0.0 ^{c}	9.3 ± 0.2 ^{b}	2.5 ± 0.6 ^{a}	5.60 ± 0.00 ^{a}	9.54 ± 0.05 ^{d}	5.19 ± 0.10 ^{a}	9.21 ± 0.16 ^{g}
Rice bran CS-*PW*	5.9 ± 0.0 ^{a}	5.9 ± 0.1 ^{a}	1.0 ± 0.0 ^{a}	8.7 ± 0.1 ^{bc}	1.5 ± 1.3 ^{a}	9.5 ± 0.1 ^{c}	9.4 ± 0.2 ^{b}	4.1 ± 0.0 ^{b}	9.7 ± 0.2 ^{c}	0.7 ± 1.2 ^{a}	6.06 ± 0.03 ^{c}	5.92 ± 0.20 ^{b}	5.82 ± 0.00 ^{d}	6.59 ± 0.10 ^{e}
Rice bran DS-*PW*	6.0 ± 0.1 ^{a}	5.9 ± 0.1 ^{a}	1.3 ± 0.1 ^{a}	8.7 ± 0.0 ^{b}	2.2 ± 0.2 ^{a}	9.4 ± 0.1 ^{bc}	9.3 ± 0.1 ^{b}	4.0 ± 0.1 ^{b}	9.6 ± 0.1 ^{c}	1.4 ± 1.3 ^{a}	5.77 ± 0.05 ^{b}	6.71 ± 0.43 ^{c}	5.66 ± 0.01 ^{c}	7.50 ± 0.14 ^{f}
Soya CS-*PL*	6.1 ± 0.1 ^{a}	6.4 ± 0.2 ^{b}	<1	8.8 ± 0.1 ^{c}	2.1 ± 0.2 ^{a}	8.0 ± 0.1 ^{a}	8.2 ± 0.1 ^{a}	5.0 ± 0.0 ^{d}	9.0 ± 0.0 ^{a}	2.1 ± 0.2 ^{a}	6.03 ± 0.00 ^{c}	4.71 ± 0.17 ^{a}	6.04 ± 0.03 ^{e}	4.55 ± 0.03 ^{c}
Soya CS-*PW*	6.0 ± 0.1 ^{a}	5.9 ± 0.1 ^{a}	1.0 ± 0.0 ^{a}	8.6 ± 0.1 ^{ab}	2.1 ± 0.2 ^{a}	9.6 ± 0.2 ^{c}	9.5 ± 0.2 ^{b}	3.7 ± 0.1 ^{a}	9.1 ± 0.1 ^{a}	1.3 ± 1.2 ^{a}	5.73 ± 0.05 ^{b}	6.01 ± 0.08 ^{bc}	5.62 ± 0.00 ^{bc}	6.08 ± 0.10 ^{d}
Soya DS-*PW*	6.0 ± 0.1 ^{a}	6.0 ± 0.0 ^{a}	<1	8.5 ± 0.0 ^{a}	1.3 ± 1.2 ^{a}	9.2 ± 0.1 ^{b}	9.3 ± 0.1 ^{b}	3.9 ± 0.1 ^{b}	9.3 ± 0.0 ^{b}	2.0 ± 0.0 ^{a}	5.57 ± 0.05 ^{a}	6.57 ± 0.23 ^{bc}	5.51 ± 0.01 ^{b}	6.84 ± 0.07 ^{e}

CS-*PW*: control sourdough fermented by *P. freudenreichii* DSM 20271 and *W. confusa* A16 mixed culture;
DS-*PW*: dextran-enriched sourdough fermented by *P. freudenreichii* DSM 20271 and *W. confusa* A16 mixed culture.
Different superscript letters in the same column indicate statistical significance ($p < 0.05$).

[a] *Bacillus cereus* cell density is very low and the contamination may be unevenly distributed in the raw materials, consequently there is large variation among the biological replicates (n = 3). Presumptive *Bacillus* spp. cell density was <3 log cfu/g in all samples after 24 h of fermentation.

[b] CS-*PL*: control sourdough fermented by *P. freudenreichii* DSM 20271 and *L. brevis* ATCC 14869 mixed culture;

Table 3
Amount of organic acids, free sugars, dextran, and vitamin B12 in sourdoughs after 24 h of fermentation.

	Acids (% dry matter)			Sugars (% dry matter)		Dextran (% dry matter)	Vitamin B12 (μg/100 g fresh weight)
	Lactic acid	Acetic acid	Propionic acid	Sucrose	Fructose		
Rice bran CS-*PL*[a]	0.90 ± 0.01 ^{d}	0.03 ± 0.00 ^{ab}	0.13 ± 0.01 ^{b}	5.74 ± 0.19 ^{a}	nd	nd	6.56 ± 0.20 ^{ab}
Rice bran CS-*PW*	0.79 ± 0.01 ^{b}	0.04 ± 0.00 ^{b}	0.12 ± 0.00 ^{ab}	nd	2.91 ± 0.05 ^{b}	2.16 ± 0.05 ^{b}	7.66 ± 0.34 ^{abc}
Rice bran DS-*PW*	0.88 ± 0.01 ^{cd}	0.04 ± 0.00 ^{b}	0.21 ± 0.01 ^{c}	nd	7.75 ± 0.25 ^{d}	5.75 ± 0.21 ^{c}	7.92 ± 0.38 ^{bc}
Soya CS-*PL*	0.73 ± 0.02 ^{a}	0.03 ± 0.00 ^{a}	0.10 ± 0.01 ^{a}	2.98 ± 0.05 ^{b}	nd	nd	6.20 ± 0.35 ^{a}
Soya CS-*PW*	0.74 ± 0.01 ^{a}	0.12 ± 0.01 ^{c}	0.19 ± 0.01 ^{c}	nd	1.35 ± 0.03 ^{a}	1.34 ± 0.03 ^{a}	9.13 ± 0.86 ^{c}
Soya DS-*PW*	0.85 ± 0.00 ^{c}	0.14 ± 0.01 ^{d}	0.29 ± 0.01 ^{d}	nd	6.56 ± 0.32 ^{c}	5.62 ± 0.15 ^{c}	8.91 ± 0.26 ^{c}

Different superscript letters in the same column indicate statistical significance ($p < 0.05$).
[a] See Table 2 for sample codes.

highest B12 content was found in soya DS-*PW* and CS-*PW* (8.9–9.1 μg/100 g fresh weight) followed by rice bran DS-*PW* and CS-*PW* (7.7–7.9 μg/100 g) (Table 3). Relatively lower levels of B12 (6.2–6.6 μg/100 g) were produced in soya and rice bran CS-*PL*. As expected, the same trend in terms of B12 content was observed in sourdough breads accordingly (Table 4). Soya DSB-*PW* and CSB-*PW* (4.8–4.9 μg/100 g fresh weight) were ranked first, followed by rice bran DSB-*PW* and CSB-*PW* (3.8 μg/100 g). Soya and rice bran CSB-*PL* contained the least B12 (3.0–3.6 μg/100 g).

3.4. Volume, texture, and mould-free shelf life of breads

Baking loss was approximately 12% for all breads (Table 4). The substitution of wheat flour with 18.5% soya flour and rice bran resulted in a significant reduction of bread-specific volume (23% and 16%, respectively) and an increase in crumb hardness (51% and 20%, respectively) compared to the wheat control bread. Irrespective of the flours used, the addition of CS-*PL* (without dextran) did not change the loaf volume or crumb hardness compared to the soya or rice bran controls. In contrast, soya and rice bran CS-*PW* increased the loaf-specific volume by 7% and 12% compared to the soya and rice bran controls, respectively. Soya and rice bran CS-*PW* exhibited comparable crumb hardness as the wheat control bread. Including soya and rice bran DS-*PW* delivered a greater improvement in the bread-specific volume by 14% and 19% compared to the soya and rice bran controls, respectively, and concurrently a significantly lower crumb hardness than that of wheat control. The different values of crumb hardness persisted during the storage period of 4 d.

Mould growth was detected on the fifth storage day at room temperature on sliced and whole breads of wheat, soya, and rice bran control (Supplementary Material Fig. S1). The estimated percentage of moulded surface in these breads was 2.5–5% and continued growing to 25–40% after 9 d of storage (Supplementary Material Fig. S2). Contamination on soya and rice bran control sourdough breads (CSB-*PL* and CSB-*PW*) appeared on the sixth day (approximately 5% of the surface area of bread loaves and slices) and increased to 25% on the ninth day. Rice bran DSB-*PW* and soya DSB-*PW* showed mould spoilage on days 7 and 9, respectively.

3.5. Sensory characteristics of breads

No significant effects of test session or interaction terms (i.e., panellist * session or sample * session) were found in the sensory data, confirming its reliability. The flavour profiles of rice bran-containing breads are shown in Fig. 2A. Rice bran control bread was differentiated from all rice bran sourdough breads by its higher intensities of cooked rice smell and taste. Adding control sourdough (CS-*PL* or CS-*PW*) to the bread formulas significantly increased sour smell, sour taste, and aftertaste compared to rice bran control bread without sourdough (Supplementary Material Table S3). Remarkably, utilizing dextran-enriched sourdough (DS-*PW*) significantly reduced the sour taste and aftertaste while increasing the cheesy smell and cheesy taste of the bread when compared with control sourdough breads. Moreover, using purified dextran-supplemented CS-*PW* (Type 10 bread in section 2.7.2) resulted in the lowest intensity ratings of all evaluated smells and tastes among sourdough breads. A similar trend was observed in soya-containing breads (Fig. 2B). Soya control bread was characterized by the highest intensity of beany smell. Soya DSB-*PW* exhibited lower intensity ratings of odours (beany, sour, and overall smell) and tastes (beany taste, sour taste, and aftertaste) compared to its counterpart soya CSB-*PW*. The only exception was cheesy taste, which was perceived the strongest in soya DSB-*PW*. The pure dextran-supplemented soya CSB-*PW* (Type 10 bread) showed the lowest intensities of smells and tastes among the sourdough breads.

The mouthfeel characteristics of soya and rice bran breads are summarized in Fig. 3. Breads with CS-*PL* and CS-*PW* were assessed as

Table 4
Volume, textural properties, and vitamin B12 content of breads.

	Sp. volume (mL/g)	Hardness day1 (g)	Hardness day 4 (g)	Baking loss (%)	Moisture loss during freeze drying (%)	Vitamin B12[a] (μg/100 g fresh weight)
Wheat control	4.37 ± 0.10 ^{f}	125.02 ± 15.07 ^{b}	229.65 ± 25.50 ^{cd}	12.5 ± 0.20 ^{c}	45.12 ± 0.79 ^{a}	
Rice bran control	3.67 ± 0.03 ^{c}	150.63 ± 25.08 ^{c}	252.17 ± 30.10 ^{d}	11.7 ± 0.24 ^{ab}	51.98 ± 0.89 ^{c}	
Rice bran CSB-*PL*[b]	3.53 ± 0.05 ^{b}	145.34 ± 16.78 ^{c}	276.89 ± 33.04 ^{e}	11.4 ± 0.30 ^{a}	52.29 ± 3.14 ^{c}	3.58 ± 0.17 ^{b}
Rice bran CSB-*PW*	4.11 ± 0.06 ^{e}	117.40 ± 15.54 ^{b}	207.01 ± 33.56 ^{bc}	12.4 ± 0.18 ^{cd}	47.48 ± 0.04 ^{ab}	3.81 ± 0.24 ^{bc}
Rice bran DSB-*PW*	4.37 ± 0.12 ^{f}	91.70 ± 11.80 ^{a}	172.05 ± 21.45 ^{a}	12.9 ± 0.34 ^{d}	46.66 ± 1.21 ^{a}	3.81 ± 0.15 ^{bc}
Soya control	3.35 ± 0.09 ^{a}	189.14 ± 32.05 ^{d}	288.47 ± 40.46 ^{e}	12.1 ± 0.29 ^{bc}	47.65 ± 1.50 ^{ab}	
Soya CSB-*PL*	3.32 ± 0.05 ^{a}	187.41 ± 29.54 ^{d}	299.03 ± 33.92 ^{e}	11.6 ± 0.21 ^{a}	51.27 ± 0.10 ^{bc}	2.98 ± 0.33 ^{a}
Soya CSB-*PW*	3.60 ± 0.07 ^{bc}	130.53 ± 19.86 ^{b}	238.04 ± 33.62 ^{d}	11.7 ± 0.35 ^{ab}	48.50 ± 0.86 ^{ab}	4.87 ± 0.58 ^{c}
Soya DSB-*PW*	3.81 ± 0.09 ^{d}	101.48 ± 13.54 ^{a}	186.52 ± 23.38 ^{ab}	12.4 ± 0.21 ^{c}	46.77 ± 1.12 ^{a}	4.81 ± 0.30 ^{c}

Different superscript letters in the same column indicate statistical significance ($p < 0.05$).
[a] Vitamin B12 based on bread fresh weight was calculated as: B12 dry weight × (1-moisture loss during freeze drying%).
[b] CSB-*PL*, control sourdough bread (fermented by *P. freudenreichii* and *L. brevis*); CSB-*PW*, control sourdough bread (fermented by *P. freudenreichii* and *W. confusa*); DSB-*PW*, dextran-enriched sourdough bread (fermented by *P. freudenreichii* and *W. confusa*).

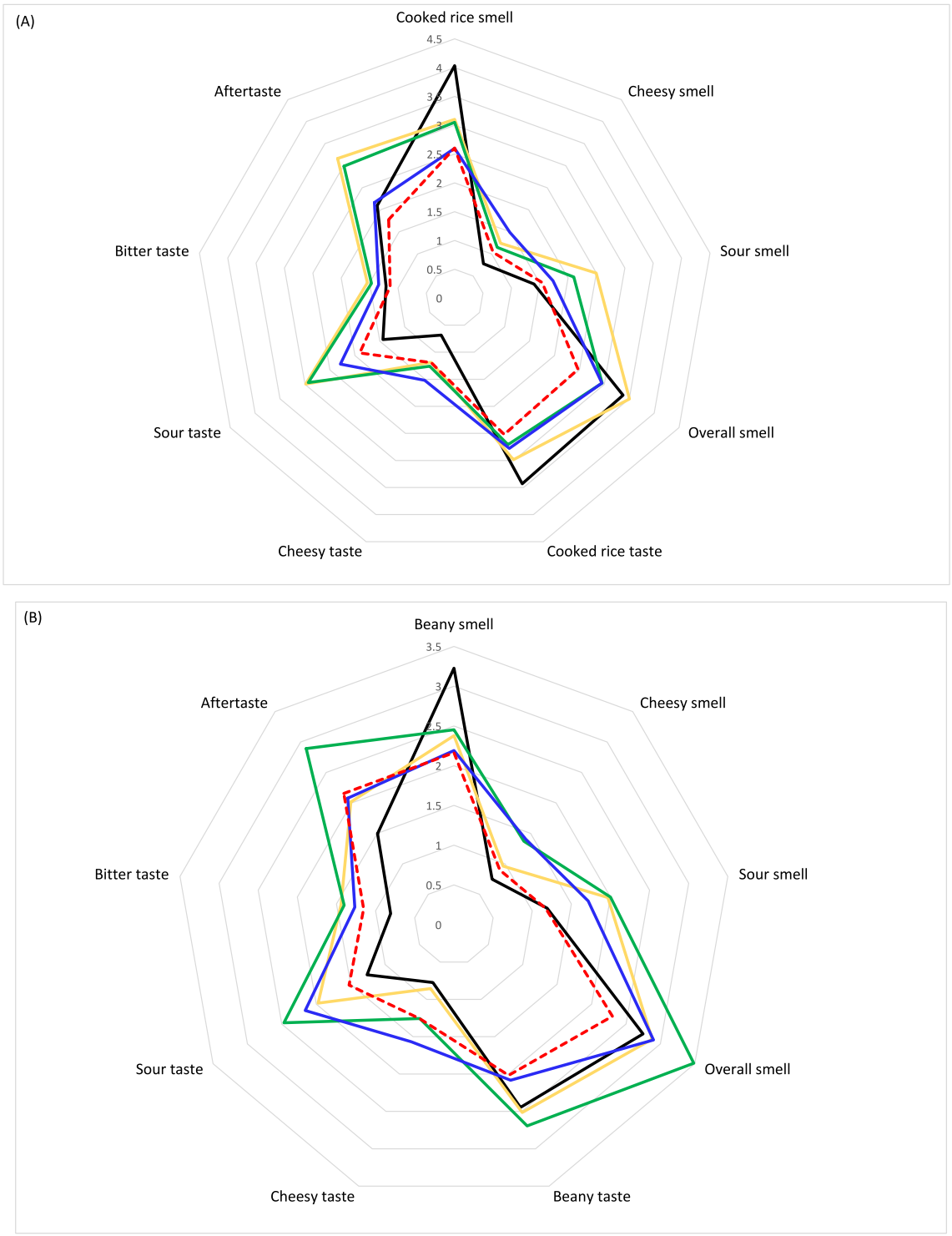

Fig. 2. Flavour profiling (ratings from 0 to 10) of rice bran (A) and soya (B) breads. The solid black line represents rice bran or soya control bread, the solid yellow line represents rice bran or soya CSB-*PL*, the solid green line represents rice bran or soya CSB-*PW*, the solid blue line represents rice bran or soya DSB-PW, and the red dashed line represents rice bran or soya CSB-*PW* bread with added pure dextran (see Table 5 for sample codes). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

similar in texture to the soya or rice bran control. However, breads containing DS-*PW* received significantly lower ratings in crumb hardness and higher ratings in moistness and cohesiveness compared to the soya or rice bran control. In addition, breads with purified dextran-enriched CS-*PW* were perceived as having the lowest crumb hardness and the highest moistness and cohesiveness.

3.6. Analysis of bread volatile flavour compounds

The identified volatile flavour compounds and their retention times, peak areas, possible origins, and aroma descriptions are shown in Table 5. There were 51 volatile components identified by HS-SPME-GC-MS in rice bran (sourdough) breads. Most of the detected aroma compounds were typical lipid oxidation products possessing green, grassy,

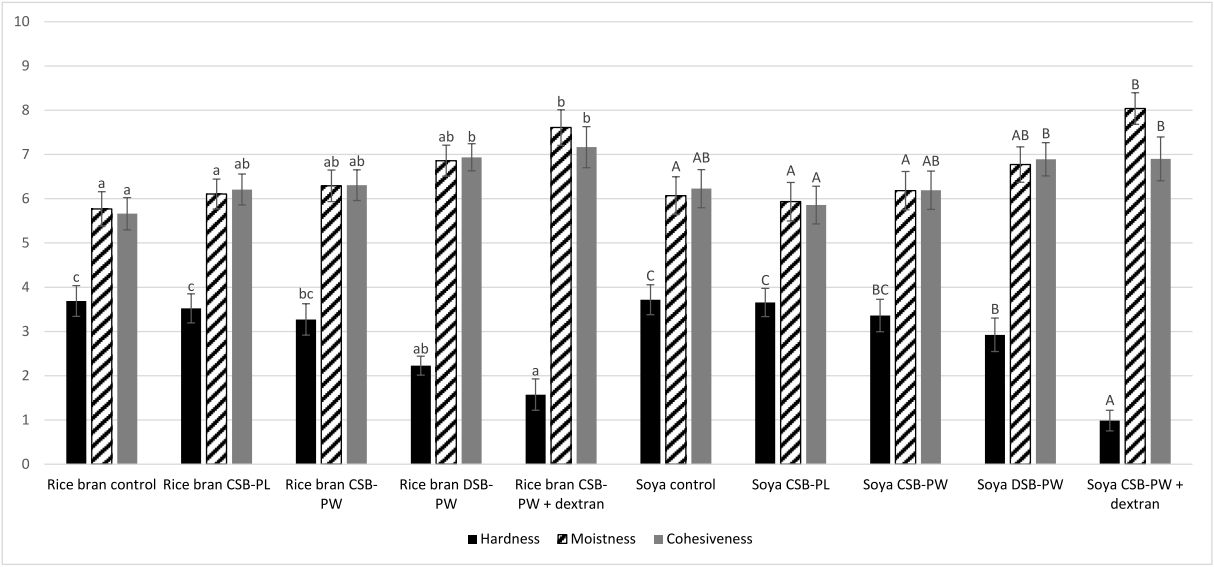

Fig. 3. Texture and mouthfeel profiling (ratings from 0 to 10) of rice bran and soya breads (see Table 5 for sample codes). Different lowercase letters indicate significant difference ($p < 0.05$) among rice bran breads; the uppercase letters indicate significant difference ($p < 0.05$) among soya breads.

fatty, or rancid odour notes (or combinations thereof). In general, these compounds were present at lower concentrations (or peak areas) in sourdough breads compared to the rice bran control, particularly in DSB-*PW* and purified dextran-supplemented CSB-*PW*. For example, the peak areas of hexanal, heptanal, and (*E*,*E*)-2,4-decadienal in DSB-*PW* and dextran-supplemented CSB-*PW* were reduced by 20–50% and 24–36%, respectively, compared to the rice bran control bread. A few exceptions included 1-hexanol, 3,5-octadien-2-ol, 2-pentylfuran, and nonanoic acid, which were found at higher levels in sourdough breads than in the rice bran control. Notably, DSB-*PW* exhibited the highest levels of cheesy odorants (e.g. propionic, 2-methylpropanoic, and isovaleric acids) among all bread types. CSB-*PL* showed an increased level of 3-hydroxy-2-butanone (buttery, creamy) when compared with others. In addition, pure dextran-supplemented CSB-*PW* showed decreased levels of nearly all fermentation-originated aroma compounds compared to CSB-*PW* without external added dextran. The Maillard reaction products conferring roasted notes, such as methylpyrazine and 2,6-dimethylpyrazine, were more apparent in DSB-*PW*.

A similar trend was observed in the volatile profiles of soya (sourdough) breads. A total of 42 compounds were identified. The green and fatty odour compounds that originated from lipid oxidation were also found in soya breads but with less variety and generally lower levels than in rice bran breads. However, two additional compounds with a mushroom-like odour (i.e. 1-octen-3-ol and 3-octanol) were present in soya breads. All lipid oxidation-derived compounds were decreased in sourdough breads compared to the soya control, except for nonanoic acid. The highest reduction was obtained in DSB-*PW* (e.g., the peak areas of hexanal, heptanal, (*E*)-2-heptenal, (*E*,*E*)-2,4-decadienal, 1-hexanol, 2-pentylfuran, and 3,5-octadien-2-one were reduced by 50–60% compared to the soya control) followed by pure dextran-supplemented CSB-*PW* (30–60% reduction). Sourdough breads were characterized by high levels of carboxylic acids and other compounds formed through the fermentation processes. Among the sourdough breads, DSB-*PW* exhibited the highest levels of acetic and propionic acids, whereas CSB-*PL* exhibited the lowest levels of these acids.

4. Discussion

This study investigated whether sufficient levels of active B12 and dextran can be produced concurrently in rice bran and soya flour via mixed fermentation using *P. freudenreichii DSM 20271* and *W. confusa* A16 to improve the shelf life and textural and sensory properties of *in situ* B12-fortified soya or rice bran breads.

Native untreated soya flour or rice bran can be contaminated with unwanted pathogens, which may pose a potential hazard to the microbial quality of food products. Under the conditions of this study, the presence of LAB was able to inhibit the growth of potential pathogens. The inhibitory effect was more pronounced in fermented samples containing *W. confusa* A16 and achieved acceptable levels of *Enterobacteriaceae* ($\leq$4 log cfu/g) and *B. cereus* ($\leq$2 log cfu/g) (EFSA BIOHAZ Panel, 2017). In addition to lower pH, the possible mechanisms of action against pathogenic microorganisms include competitive exclusion and secretion of antimicrobial compounds, including lactic, propionic, and acetic acid (Hossain, Sadekuzzaman, & Ha, 2017).

W. confusa dextransucrase catalyses the cleavage of the glycosidic bond of sucrose and the transfer of the glucosyl unit to a dextran chain for polymerization while releasing fructose (Monsan et al., 2001). The sucrose in soya or rice bran sourdoughs containing *W. confusa* A16 (i.e., DS-*PW* and CS-*PW*) was completely consumed and the liberated fructose was nearly unused during the 24 h of fermentation. In this study, the initial pH of soya flour and rice bran was approximately 6.8 (optimal for LAB growth and dextransucrase production) and decreased to 5.6–6.0 (optimal for dextransucrase activity) (Vettori, Blanco, Cortezi, Lima, & Contiero, 2012) after 24 h of fermentation. This may explain the high sucrose to dextran conversion in soya flour and rice bran, which amounts to 85% and 72% of the theoretical yields, respectively. In addition, the absence of maltose in soya flour and rice bran may also explain the high conversion rate, as maltose (e.g. in wheat) is a strong acceptor sugar for dextransucrase and competes with dextran production (Molina, Cioci, Moulis, Séverac, & Remaud-Siméon, 2021).

The high pH of soya flour and rice bran provides favourable conditions for the growth of *P. freudenreichii* (optimal pH approximately 7.0) and B12 biosynthesis (pH 6.5–7.0) (Piwowarek, Lipińska, Hać-Szymańczuk, Kieliszek, & Ścibisz, 2018). Higher quantities of propionic and acetic acids were obtained in soya flour or rice bran fermented by the mixed culture of *P. freudenreichii* with *W. confusa* (DS-*PW* and CS-*PW*) compared to *L. brevis* (CS-*PL*). *P. freudenreichii* can utilize lactate, glucose, and fructose, with lactate being the most preferred carbon source, resulting in the production of propionate, acetate, and CO_2 (2:1:1) (Piwowarek, Lipińska, Hać-Szymańczuk, Bzducha-Wróbel, & Synowiec, 2018). The glucose liberated from sucrose by *W. confusa* dextransucrase activity likely facilitated the production of propionic and

Table 5
List of identified bread volatile compounds isolated by HS-SPME-GC-MS.

RT (min)	Compound name	Possible origins[a]	Odour description	Rice bran control	Rice bran CSB-*PL*[b]	Rice bran CSB-*PW*	Rice bran DSB-*PW*	Rice bran CSB-*PW* + dextran	Soya control	Soya CSB-*PL*	Soya CSB-*PW*	Soya DSB-*PW*	Soya CSB-*PW* +dextran
				Area (Ab × s) × 10^6									
3.57	Ethanol	Fermentation	Alcohol	145.2 ± 7.7[c]	206.8 ± 84.5	127.2 ± 10.1	183.5 ± 96.1	136.8 ± 17.8	141.9 ± 17.7	165.1 ± 48.5	123.2 ± 29.0	168.1 ± 60.8	125.4 ± 23.6
5.89	1-Propanol	Fermentation	Alcohol, fermented, musty			2.0 ± 0.2	2.3 ± 0.6	1.4 ± 0.1		0.3 ± 0.0	1.8 ± 0.7	1.6 ± 0.1	1.4 ± 0.5
6.39	2,3-Butanedione	Fermentation	Creamy, buttery				0.7 ± 0.2			1.0 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.5 ± 0.0
7.94	2-Methyl-1-propanol/ Isobutanol	Fermentation	Ethereal, winey, alcohol	3.2 ± 0.1	7.1 ± 0.7	7.3 ± 1.2	10.5 ± 4.2	5.7 ± 1.1	3.8 ± 0.9	10.8 ± 2.0	6.6 ± 2.6	10.2 ± 2.0	10.2 ± 0.4
8.69	Acetic acid	Fermentation	Vinegar, sour	3.1 ± 1.6	11.8 ± 6.6	19.1 ± 0.1	20.6 ± 3.4	13.2 ± 1.2	2.1 ± 0.4	11.8 ± 3.0	26.0 ± 5.7	40.0 ± 10.6	24.0 ± 4.5
9.89	Propanoic acid ethyl ester	Fermentation	Pineapple-like		0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0.2	0.6 ± 0.2		0.6 ± 0.4	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.2
11.49	3-hydroxy-2-butanone	Lipid oxidation/ Fermentation	Sweet, creamy buttery,	2.4 ± 2.9	4.0 ± 2.0	2.2 ± 0.1	2.1 ± 1.5	1.7 ± 0.1	4.6 ± 0.4	6.4 ± 4.4	2.1 ± 0.5	3.3 ± 1.0	1.5 ± 0.4
11.76	3-Methyl-1-butanol	Lipid oxidation/ Fermentation	Alcohol, fermented, fruity, whiskey	13.4 ± 2.7	11.3 ± 3.3	6.1 ± 0.4	7.2 ± 1.6	1.7 ± 0.2	12.1 ± 1.6	8.8 ± 0.3	6.2 ± 2.3	10.6 ± 1.5	7.2 ± 2.8
11.88	2-Methyl-1-butanol	Maillard reactions	Cooked, roasted		3.4 ± 0.1				6.5 ± 0.6	4.0 ± 0.6		2.9 ± 0.8	
12.12	Propanoic acid (Propionic acid)	Fermentation	Cheesy, acidic, vinegar		25.9 ± 2.3	65.1 ± 1.4	70.1 ± 3.4	39.7 ± 8.3		45.7 ± 2.8	64.5 ± 15.7	70.5 ± 7.0	63.0 ± 12.9
13.71	Hexanal	Lipid oxidation	Green, fatty, grassy	7.5 ± 1.1	8.4 ± 0.3	6.8 ± 0.2	5.9 ± 0.7	5.7 ± 1.6	6.3 ± 0.3	4.0 ± 0.2	3.5 ± 0.8	2.6 ± 0.6	2.8 ± 0.5
14.00	2-Methylpropanoic acid	Fermentation	Cheesy	2.5 ± 0.1	3.9 ± 0.2	4.9 ± 0.1	5.6 ± 1.2	3.0 ± 0.7	2.5 ± 0.1	4.4 ± 0.4	3.1 ± 1.0	4.4 ± 0.6	3.2 ± 0.9
14.54	Methylpyrazine	Maillard reactions	Roasted, burnt	0.9 ± 0.1			1.0 ± 0.3	0.5 ± 0.0	0.7 ± 0.0	0.6 ± 0.1		1.6 ± 0.2	0.9 ± 0.2
15.11	Butanoic acid	Fermentation	Cheesy, buttery, acidic, fruity	0.7 ± 0.2	1.4 ± 0.1	1.7 ± 0.0	1.8 ± 0.1	1.0 ± 0.3	0.7 ± 0.0	1.5 ± 0.4	1.0 ± 0.2	1.3 ± 0.1	1.0 ± 0.3
16.01	Furfural	Fermentation	Sweet, almond, bread	3.8 ± 0.1		1.1 ± 0.1	4.7 ± 0.7	1.5 ± 0.1	2.3 ± 0.5	1.2 ± 0.1		1.8 ± 0.1	0.4 ± 0.2
16.28	(E)-2-Hexenal	Lipid oxidation	Green, fatty	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.0	0.5 ± 0.1	0.4 ± 0.1		
16.67	1-Hexanol	Lipid oxidation fermentation	Oily, alcohol, green	13.5 ± 3.7	21.0 ± 0.1	19.9 ± 0.2	16.6 ± 1.6	14.7 ± 5.1	9.6 ± 0.6	9.3 ± 1.2	7.1 ± 1.9	5.5 ± 1.3	6.6 ± 1.3
17.07	Isovaleric acid	Fermentation	Pungent cheesy, sweaty smell (foot)		3.4 ± 0.1	4.4 ± 0.1	4.3 ± 0.2	2.7 ± 1.0		3.9 ± 0.1	3.7 ± 1.0	4.1 ± 1.0	3.8 ± 0.8
17.27	2-methylbutanoic acid	Fermentation	Pungent cheesy, acidic, rancid	2.9 ± 0.2	1.8 ± 0.5	2.4 ± 0.1	2.2 ± 0.2	1.6 ± 0.5		2.6 ± 0.1	1.6 ± 0.5	1.4 ± 0.3	1.7 ± 0.3
17.51	Heptanal	Lipid oxidation	Fatty, green, grassy	3.2 ± 0.5	3.2 ± 0.4	2.7 ± 0.1	2.1 ± 0.3	2.3 ± 0.7	3.1 ± 0.1	2.4 ± 0.3	1.7 ± 0.4	1.3 ± 0.4	1.4 ± 0.1
17.70	2,6-dimethylpyrazine	Maillard reactions	Roasted, nutty, coffee	0.8 ± 0.2	0.7 ± 0.0	0.8 ± 0.1	1.3 ± 0.0	0.6 ± 0.2	1.5 ± 0.3	1.1 ± 0.4	0.8 ± 0.3	1.3 ± 0.4	1.0 ± 0.1
19.87	2-Pentylfuran	Lipid oxidation	Green, earthy, beany	7.3 ± 1.3	11.8 ± 0.6	10.8 ± 0.3	10.5 ± 1.3	7.9 ± 2.0	9.3 ± 0.0	6.5 ± 0.7	6.5 ± 1.0	4.1 ± 0.6	5.4 ± 0.4
20.05	(E)-2-Heptenal	Lipid oxidation/ Fermentation	Sour, green, fatty	4.8 ± 0.6	4.1 ± 0.3	4.0 ± 0.1	3.3 ± 0.4	4.3 ± 0.7	1.8 ± 0.1	1.3 ± 0.2	1.1 ± 0.2	0.9 ± 0.1	1.1 ± 0.0
20.24	1-Heptanol	Lipid oxidation	Aromatic, fatty			2.3 ± 0.1	1.7 ± 0.2	2.4 ± 0.2	1.4 ± 0.1				
20.42	1-Octen-3-ol	Lipid oxidation	Earthy, fungal, mushroom, hay-like						21.1 ± 0.8	18.6 ± 1.8	17.0 ± 2.7	15.3 ± 2.2	16.6 ± 1.1
20.47	Benzaldehyde	Lipid oxidation/ Fermentation	Almond, burnt sugar	8.5 ± 1.1	8.5 ± 0.1	8.8 ± 0.1	7.3 ± 1.4	7.7 ± 1.3					

(*continued on next page*)

Table 5 (*continued*)

RT (min)	Compound name	Possible origins[a]	Odour description	Rice bran control	Rice bran CSB-*PL*[b]	Rice bran CSB-*PW*	Rice bran DSB-*PW*	Rice bran CSB-*PW* + dextran	Soya control	Soya CSB-*PL*	Soya CSB-*PW*	Soya DSB-*PW*	Soya CSB-*PW* +dextran
20.69	6-Methyl-5-hepten-2-one	Fermentation	Citrus, fruity	3.9 ± 0.6	3.8 ± 0.2	2.4 ± 0.0	2.5 ± 0.4	2.0 ± 0.5					
20.80	2-Octanone	Lipid oxidation/ Fermentation	Earthy, grassy, woody	6.3 ± 1.9	3.5 ± 0.5	3.0 ± 0.1	4.1 ± 1.2	2.6 ± 0.7					
20.86	3-Octanol	Lipid oxidation	Earthy, mushroom						9.2 ± 0.4	6.5 ± 0.8	6.3 ± 1.0	5.2 ± 1.0	6.4 ± 0.1
21.13	DL-Limonene	Fermentation	Citrus, lemon	3.9 ± 0.9	4.7 ± 0.5	4.6 ± 0.1	3.7 ± 0.5	3.3 ± 0.8	3.0 ± 0.0	2.0 ± 0.4	2.1 ± 0.3	1.4 ± 0.4	1.9 ± 0.0
21.27	1-Methyl-4-(1-methylethyl) benzene	Fermentation	Woody, citrus, lemon	1.0 ± 0.3	1.5 ± 0.2	1.6 ± 0.1	0.9 ± 0.1	1.4 ± 0.3					
21.73	Hexanoic acid	Lipid oxidation/ Fermentation	Sour, cheesy	5.4 ± 1.1	10.4 ± 0.7	11.7 ± 0.2	11.7 ± 1.0	8.3 ± 2.6		2.3 ± 0.5	2.4 ± 0.2	1.7 ± 0.5	2.5 ± 0.1
22.25	(E,E)-2,4-Heptadienal	Lipid oxidation	Green, fatty, oily	2.5 ± 0.6	3.1 ± 0.2	3.1 ± 0.7	2.9 ± 0.2	2.6 ± 0.5					
22.58	3-Ethyl-2-methyl-1,3-hexadiene		A major aroma compound in sweet corn	1.6 ± 0.5	1.5 ± 0.1	1.5 ± 0.3	1.5 ± 0.2	2.1 ± 0.5		0.4 ± 0.0		0.4 ± 0.1	
22.79	3,5-Octadien-2-ol	Lipid oxidation	Green, grassy	2.2 ± 0.9	3.3 ± 0.3	3.6 ± 0.3	3.2 ± 0.3	2.6 ± 0.6	0.9 ± 0.1	0.5 ± 0.1	0.6 ± 0.0		0.6 ± 0.0
23.53	2-Octenal	Lipid oxidation	Cereal, green, fatty, grassy	13.6 ± 2.2	15.0 ± 1.2	14.9 ± 0.4	13.0 ± 1.2	14.8 ± 3.5		6.1 ± 0.4	5.5 ± 0.6	4.2 ± 0.8	4.9 ± 0.1
23.97	3,5-Octadiene-2-one	Lipid oxidation	Fruity, fatty, Mushroom	4.0 ± 0.5	3.4 ± 0.2	3.0 ± 0.1	2.7 ± 0.2	2.3 ± 0.4					
24.16	2-Nonanone	Lipid oxidation	Green, weedy, herbal	2.6 ± 0.5	3.4 ± 0.3	3.2 ± 0.2	3.1 ± 0.4	2.6 ± 0.6	3.5 ± 0.2	2.8 ± 0.2	2.2 ± 0.1	1.9 ± 0.0	1.9 ± 0.3
24.49	Nonanal	Lipid oxidation	Rose, citrus, orange	17.4 ± 3.0	19.4 ± 1.9	18.3 ± 0.6	14.9 ± 2.1	12.4 ± 2.9	12.1 ± 0.3	7.9 ± 0.8	6.4 ± 0.6	7.4 ± 0.9	5.1 ± 0.1
24.87	3,5-Octadien-2-one	Lipid oxidation	Fruity, fatty, mushroom	7.2 ± 1.2	9.2 ± 0.8	10.1 ± 0.3	8.6 ± 0.9	8.5 ± 1.1	1.5 ± 0.2	1.0 ± 0.1	0.9 ± 0.2	0.7 ± 0.1	0.6 ± 0.0
25.31	6-Methyl-3,5-heptadien-2-one		Cinnamon-like, green, spicy	0.6 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	0.5 ± 0.0					
25.97	Dodecane	Lipid oxidation	Alkane	0.8 ± 0.2	1.0 ± 0.3	1.0 ± 0.0	1.0 ± 0.1	0.8 ± 0.2		0.6 ± 0.1		1.0 ± 0.4	1.2 ± 0.1
26.09	2-Phenylethanol	Fermentation	Floral, rose	8.3 ± 2.1	8.0 ± 1.2	5.8 ± 0.1	5.6 ± 0.9	5.1 ± 0.8	22.8 ± 0.1	8.1 ± 1.1	4.3 ± 1.9	4.2 ± 0.4	5.6 ± 0.2
26.77	Nonenal	Lipid oxidation	Fatty, green	9.7 ± 1.7	11.8 ± 0.2	14.8 ± 0.1		15.1 ± 2.1	13.2 ± 0.4	13.0 ± 0.7	10.1 ± 1.0	9.2 ± 0.3	10.1 ± 0.4
27.64	Decanal	Lipid oxidation/ Fermentation	Floral, orange	1.6 ± 0.2	2.7 ± 0.9	1.9 ± 1.1	2.3 ± 1.0	0.7 ± 0.1					
27.69	Octanoic acid	Fermentation	Fatty, rancid, cheesy						1.7 ± 0.4	2.1 ± 0.4	1.5 ± 0.4	1.4 ± 0.1	1.1 ± 0.2
28.74	2,4-Nonadienal	Lipid oxidation/ Fermentation	Fatty, nutty, leafy, fried	1.2 ± 0.1	1.3 ± 0.3	1.4 ± 0.2	1.2 ± 0.2	1.3 ± 0.2					
29.47	Delta-(4)-dodecanol	Fermentation	Floral, fruity	2.0 ± 0.3	3.1 ± 0.4	3.1 ± 0.1	2.7 ± 0.3	3.8 ± 0.5	1.7 ± 0.1	1.8 ± 0.0			
29.65	Nonanoic acid ethyl ester	Lipid oxidation	Fatty, oily, nutty	1.2 ± 0.1	1.5 ± 0.2	1.5 ± 0.1	1.6 ± 0.2	1.3 ± 0.1					
29.81	(E)-2-Decenal	Lipid oxidation	Fatty	3.4 ± 0.3	2.8 ± 0.1	2.8 ± 0.2	2.3 ± 0.1	2.6 ± 0.2					
30.47	Nonanoic acid	Lipid oxidation	Rancid	4.5 ± 2.1	10.6 ± 1.2	8.3 ± 1.8	11.2 ± 3.9	14.7 ± 2.8	3.5 ± 0.8	7.2 ± 0.7	4.0 ± 0.9	4.7 ± 1.1	4.9 ± 0.5
30.97	(E,Z)-2,4-Decadienal	Lipid oxidation/ Maillard reactions	Fatty, green, fried	16.5 ± 1.1	12.0 ± 0.6	13.6 ± 0.3	9.6 ± 0.2	12.4 ± 0.9					
31.68	(E,E)-2,4-Decadienal	Lipid oxidation	Green, fatty, citrus, fried	93.0 ± 8.2	62.6 ± 1.7	66.9 ± 1.3	48.3 ± 0.6	59.9 ± 3.9	1.6 ± 0.1	1.2 ± 0.2	1.0 ± 0.2	0.7 ± 0.1	0.9 ± 0.0
34.19	Dihydro-5-pentyl-2(3H)-furanone	Lipid oxidation	Coconut	1.4 ± 0.3	1.8 ± 0.1	1.3 ± 0.1	1.4 ± 0.2	1.4 ± 0.3	1.3 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	0.6 ± 0.1	0.7 ± 0.1

[a] Possible origins and odour descriptions are from Nedele et al. (2021) and Pétel et al. (2017).

[b] CSB-*PL*, control sourdough bread (fermented by *P. freudenreichii* and *L. brevis*); CSB-*PW*, control sourdough bread (fermented by *P. freudenreichii* and *W. confusa*); DSB-*PW*, dextran-enriched sourdough bread (fermented by *P. freudenreichii* and *W. confusa*); CSB-*PW* + dextran, control sourdough bread with added purified dextran.

[c] Results are mean values (n = 3) ± standard deviation.

acetic acids (Xie et al., 2019). This may explain the higher B12 yield in soya or rice bran sourdoughs fermented by *P. freudenreichii* and *W. confusa* than by *L. brevis*, as B12 functions as a coenzyme in *P. freudenreichii* in the production pathway of propionic acid (Piwowarek, Lipińska, Hać-Szymańczuk, Bzducha-Wróbel, & Synowiec, 2018). The B12 produced during fermentation was in the active form, with 5, 6-dimethylbenzimidazole (DMBI) as a lower ligand (confirmed by UHPLC–MS/MS analysis). It is worth nothing that we did not supplement the raw materials with any precursors (e.g. cobalt and riboflavin) important for the biosynthesis of B12 to observe the inherent B12 production capacity of *P. freudenreichii*.

The incorporation of 50% (dw) of the fermented soya flour and rice bran in bread making resulted in a substantial quantity of B12, which exhibited high stability during the bread-making process with a loss of approximately only 4%. The content of active B12 in breads containing soya or rice bran sourdoughs fermented by *P. freudenreichii* and *W. confusa* (DSB-*PW* and CSB-*PW*) was 3.8–4.9 μg/100 g fresh weight, which was higher than that of B12 naturally found in meat, poultry, eggs, and dairy products (Combs & McClung, 2017). Consuming 100 g of these sourdough breads, which is approximately 50% of the daily bread consumption in many Western countries (Eglite & Kunkulberga, 2017; Lockyer & Spiro, 2020), would meet the recommended daily requirement or adequate intake (2.4–4 μg) of vitamin B12 (EFSA NDA Panel, 2015; Institute of Medicine, 1998).

As expected, substituting wheat with 18.5% fw native soya flour or rice bran led to the deterioration of bread textural quality. When the soya or rice bran sourdough fermented by *P. freudenreichii* and *L. brevis* (CSB-*PL*, without dextran) was added, the bread volume and textural properties remained unchanged compared to the soya or rice bran control. In contrast, the *in situ* produced dextran in soya or rice bran sourdoughs fermented by *P. freudenreichii* and *W. confusa* (DSB-*PW* and CSB-*PW*) improved bread volume and crumb softness. A greater degree of improvement was achieved in DSB-*PW* (0.6% dextran, bread weight) than its control counterpart CSB-*PW* (0.1–0.2% dextran), indicating a positive dose-response effect of dextran on bread texture (Wang et al., 2020). The instrumental measured texture parameters highly correlated with the sensory perception of mouthfeel attributes. The perceived crumb softness, moistness, and cohesiveness were the highest in soya or rice bran DSB-*PW*. The mouthfeel-enhancing effect was further confirmed by adding purified dextran to soya or rice bran CSB-*PW*. The mechanisms of dextran functionality are likely linked to its high water-binding capacity and intermolecular interactions (e.g. hydrogen bonding between dextran and gluten proteins that reinforces the gluten network) (Wang et al., 2021). Moreover, soya or rice bran DSB-*PW* had a shelf life prolonged by 2–4 days compared to the control breads. The mould-free shelf life positively correlated with the concentrations of antifungal metabolites of sourdough, in particular propionic acid (Quattrini et al., 2019).

The soya and rice bran control breads were characterized by a high score of beany and cooked rice smell and taste, respectively. The headspace volatile analysis showed a plethora of lipid-oxidation products in these breads. Considering the high fat content (20%) of soya flour or rice bran, lipid oxidation likely represents a major flavour formation route that produces beany or grassy odour components. The higher abundance of lipid-derived volatiles in rice bran bread than in soya bread was possibly due to lipid-modifying enzymes (e.g. lipase, lipoxygenase) present in the unfermented raw materials (Pal Singh & Singh Sogi, 2016). Using sourdoughs fermented by a mixed culture of *P. freudenreichii* and LAB (CSB-*PL*, CSB-*PW*, and DSB-*PW*) resulted in appreciable decreases in beany or green-associated volatile compounds measured by GC-MS, corresponding to a lower perceived intensity of beany or cooked rice smell than in the soya or rice bran control bread. The decrease of these unpleasant odorants by sourdough fermentation was probably due to enzyme activity (such as aldehyde dehydrogenase), which converted the key aroma compounds contributing to beany or green off-odour notes (e.g. hexanal, (*E*,*E*)-2,4-decadienal, 1-hexanol, and 2-pentyfuran) into the corresponding alcohols or acids (Nedele, Gross, Rigling, & Zhang, 2021). On the other hand, the sourdough breads (CSB-*PL*, CSB-*PW*, and DSB-*PW*) were perceived as more sour and cheesy with a stronger aftertaste. The headspace analysis revealed an increased amount of sour or cheesy-associated (or both) volatiles in the sourdough breads (e.g. acetic acid, propionic acid, isovaleric acid, and hexanoic acid derived from the metabolic activity of the starter cultures) (Pétel, Bernard, & Carole, 2017).

Dextran produced *in situ* had a significant impact on the flavour properties of soya or rice bran sourdough breads. DSB-*PW* (fermented by *P. freudenreichii* and *W. confusa* with sucrose addition) exhibited the strongest cheesy flavour among all the tested breads, consistent with its higher propionic acid content. Despite the higher level of acidity, the soya or rice bran DSB-*PW* was rated as less intense in beany or cooked rice smell and taste, sour smell and taste, and aftertaste compared to its control counterpart CSB-*PW* (fermented by *P. freudenreichii* and *W. confusa* without sucrose addition). DSB-*PW* contained more residual fructose that may serve as precursors for the Maillard reaction and contribute to the sweet taste of the bread. Sweet taste is generally suppressive of other basic tastes at high concentrations (Keast & Breslin, 2003). However, the panel did not discriminate the sweetness intensity of DSB-*PW* from CSB-*PW* and both breads were described as having a mild sweet taste (data not shown). The synthesized dextran was responsible for the reduced aroma and taste perception in DSB-*PW*. This was evidenced by adding purified dextran in soya or rice bran CSB-*PW* (CSB-*PW* + dextran), which exhibited a suppressive effect on all evaluated smells and tastes compared to plain CSB-*PW*.

The flavour-masking effect of dextran is concentration-dependent (Wang et al., 2020). DSB-*PW* and pure dextran-supplemented CSB-*PW*, both of which contained 0.6% (bread weight) dextran, exhibited a significant decrease of the detected representative green or fatty odorants and consequently less perceived beany or cooked rice flavour compared to CSB-*PW* (0.1–0.2% dextran) and CSB-*PL* (without dextran). The presence of dextran at a concentration higher than its critical overlap concentration (a threshold at or above which flavour perception is modified; 0.43% in terms of *W. confusa* A16 dextran (Wang et al., 2020)) resulted in significant changes in the oral texture of the breads (e.g. cohesiveness and softness). This may lead to an altered breakage function of the breads during chewing and consequently different flavour-release kinetics (Tournier, Sulmont-Rossé, & Guichard, 2007). Furthermore, as a linear long-chain polymer, the produced dextran may entrap the flavour compounds through molecular interactions such as hydrogen bonding, van der Waals interactions, steric interactions, or combinations thereof (Tournier et al., 2007). This flavour-binding phenomenon may explain the lower levels of volatile compounds detected by HS-SPME-GC–MS. Further investigation is needed to measure the molecular interactions between dextran and flavour volatile compounds to provide detailed mechanistic insight.

5. Conclusions

Mixed-culture fermentation of *P. freudenreichii* and *W. confusa* was successfully applied to simultaneously produce substantial quantities of dextran and B12 in soya flour or rice bran, resulting in fortified breads with improved texture quality and less off-flavours compared to the control soya or rice bran (sourdough) breads. Our findings suggest that the mixed fermentation led to the formation of organic acids that prolonged the microbial shelf life and altered flavour properties of bread products. Dextran produced *in situ* was effective in reducing or masking the sour taste, aftertaste, and beany off-flavours. This was consistent with the reduction in typical green or fatty volatile components. Taken together, the mixed-culture fermentation by *P. freudenreichii DSM 20271* and *W. confusa A16* represents a promising clean-label method for delivering texture-enhancing, flavour-masking, and antimicrobial compounds, potentially improving consumer acceptability of vitamin B12-fortified plant-based products.

CRediT authorship contribution statement

Yaqin Wang: Methodology, Investigation, Formal analysis, Writing – original draft. **Chong Xie:** Methodology, Investigation. **Marjo Pulkkinen:** Methodology, Validation. **Minnamari Edelmann:** Investigation. **Bhawani Chamlagain:** Methodology, Supervision, Validation. **Rossana Coda:** Methodology, Supervision. **Mari Sandell:** Supervision, Validation. **Vieno Piironen:** Supervision, Validation. **Ndegwa Henry Maina:** Supervision, Funding acquisition. **Kati Katina:** Supervision, Funding acquisition.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113407.

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