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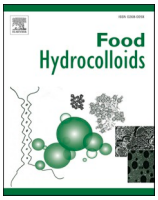

Masking off-flavors of faba bean protein concentrate and extrudate: The role of *in situ* and *in vitro* produced dextran

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ABSTRACT

This study produced dextran both *in situ* by fermentation with *Weissella confusa* A16 and *in vitro* by isolated *W. confusa* A16 dextransucrase enzyme, and investigated its effects on the flavor properties of faba bean protein concentrate (FPC) and the corresponding extrudates prepared by high moisture extrusion. Descriptive sensory profiling revealed that the FPC and extrudates had an intense pea odor and flavor, bitter taste, and astringency. Partial least squares regression analysis suggested that the pea flavor was related to the presence of lipid-oxidation products such as hexanal, heptanal, and nonanal, whereas the bitterness and astringency were likely linked to vicine, convicine, condensed tannins, and arginine. Fermentation under optimized conditions resulted in low acid formation and sufficient dextran production (1.2% end-product basis), which was effective in masking pea and bitter off-notes and enhancing pleasant flavors in FPC (sweet and fruity) and extrudates (sweet and umami). The sweetness was related to fructose produced during fermentation, and the fruity odor was linked to the generated isoamyl isovalerate and ethyl acetate. The masking effect on pea and bitter off-notes was further confirmed by adding enzymatically synthesized dextran in FPC (1.2% dextran) and extrudates (1% dextran). Overall, fermentation with *W. confusa* A16 or addition of the enzymatically produced dextran showed potential for masking off-flavors of faba bean-based ingredients and extruded meat alternatives. Furthermore, the fermentation method was associated with nutritional benefits (reduction of anti-nutritional factors, e.g., condensed tannins and verbascose) and the generation of flavor compounds/precursors (e.g., esters and free amino acids).

1. Introduction

The FAO's Strategic Framework 2022–2031 focuses on the transformation to more efficient, inclusive, resilient and sustainable agri-food systems for improved production, nutrition, and environment, along with a better life, leaving no one behind. This transition requires shifting diets towards more plant-based food, which is a more sustainable option due to the lower carbon footprint and lesser environmental effects (e.g., air and water pollution, land resource, global warming, biodiversity loss, and soil acidification) than the livestock sector (Mazac et al., 2022). Legumes have the potential to contribute to more sustainable agri-food systems. For example, faba bean (*Vicia faba* L.) improves soil fertility through nitrogen fixation and facilitating diversification of agro-ecosystems (Stagnari, Maggio, Galieni, & Pisante, 2017). It is an important crop in Europe, accounting for 28.9% of the global production between 2015 and 2020 (FAOSTAT, 2022). However, it is currently underutilized for human consumption. In recent years, Western countries have become increasingly interested in faba bean as an alternative cost-effective and resource-efficient protein source (Augustin & Cole,

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Abbreviations

Cfu	colony-forming unit
Dextran-POS	dextran positive;
Dextran-NEG	dextran negative;
EPM	enzyme production medium
FAAs	free amino acids
FFAs	free fatty acids
FPC	faba bean protein concentrate
HME	High moisture extrusion
HPAEC-PAD	high-performance anion-exchange chromatography with pulsed amperometric detection
HS-SPME-GC-MS	head-space solid-phase micro-extraction GC-MS
LAB	lactic acid bacteria
PLSR	Partial least squares regression
TTA	total titratable acidity
UHPLC	ultra-high performance liquid chromatography

2022). Faba bean has a high content of proteins (25%–40%), dietary fiber (11%–30%), vitamins, minerals, and bioactive components such as polyphenols (Jezierny, Mosenthin, & Bauer, 2010).

Protein-rich ingredients derived from faba bean, e.g., protein concentrate (51%–66%), are promising for making plant-based meat alternatives with a fibrous structure, achieved by using high moisture extrusion (HME) technology (Saldanha do Carmo et al., 2021; Ferawati et al., 2021; Tuccillo, Kantanen, et al., 2022). HME (moisture content >40%) is by far the most frequently used and established technique in the manufacture of meat alternatives. HME generally involves three essential stages: mixing, melting (by pressure and heat) and cooling (using a long cooling die). The final stage allows the proteins to align in the direction of the flow, forming a layered meat-like structure.

Using faba bean-based ingredients in food processes, such as extrusion processing, faces challenges associated with undesirable flavors, e.g., beany flavor, bitter taste, and strong aftertaste (Tuccillo, Kantanen, et al., 2022). The beany flavor is associated with the oxidation of polyunsaturated fatty acids via non-enzymatic (autoxidation) and/or enzymatic (lipoxygenase) pathways (Roland, Pouvreau, Curran, van de Velde, & de Kok, 2017). Lipid oxidation leads to formation of volatile and nonvolatile constituents causing off-flavors in final products (Wang, Tuccillo, et al., 2022). The intense bitterness and astringency in faba bean ingredients and products are likely due to the presence of free phenolic compounds, vicine and convicine (Tuccillo, Kantanen, et al., 2022). Bitter is innately disliked and could elicit a "warning sensation" that negatively affects consumers' responses (Pierguidi, Spinelli, Prescott, Monteleone, & Dinnella, 2023). Although flavor is a key factor affecting the competitiveness and consumer choice of plant-based food (Weinrich, 2019), scientific studies on flavor improvement or off-flavor removal of faba bean-based ingredients and meat alternatives are largely lacking in the literature. In current plant-based industrial applications, sugars or flavorings are most commonly used as masking agents (Grasso, Alonso-Miravalles, & O'Mahony, 2020; Montemurro, Pontonio, Coda, & Rizzello, 2021). However, the use of additives or flavoring agents has promoted consumer concerns about potential adverse health effects. Indeed, "clean label" (familiar ingredients, shorter ingredient lists, and fewer additives) is one of the biggest trends in response to consumer demand for natural products (Maruyama, Streletskaya, & Lim, 2021).

Dextrans have been extensively studied in recent years as a natural replacement of commercial hydrocolloids. Dextrans are produced by extracellular dextransucrase (EC 2.4.1.5), which is released from lactic acid bacteria (LAB) and catalyzes the hydrolysis of sucrose and polymerization of the released glucosyl units into dextran (Monsan et al., 2001). Dextrans are therefore produced either *in situ* by cultivating LAB strains in sucrose-enriched growth medium (fermentative synthesis) or *in vitro* by using cell-free supernatant containing dextransucrase (enzymatic synthesis) (Leemhuis et al., 2013). Dextrans produced by *Weissella* strains, which exhibit a linear structure (e.g., 97% α-(1 → 6) main linkage and 3% α-(1 → 3) side chains) and a high molecular weight ($>10^3$ kDa), have been demonstrated as superior texturing agents in bakery products (Wang et al., 2021). Furthermore, dextran synthesized by *W. confusa* A16 has shown a clear flavor-masking effect on beany note, bitter taste, and aftertaste in legume-enriched or wholegrain bread, according to our latest studies (Wang, Trani, et al., 2020; 2022c).

In the present study, we hypothesized that dextran produced *in situ* by fermentation with *W. confusa* A16 or *in vitro* by isolated dextransucrase enzyme (from *W. confusa* A16) can function as a masking agent for off-flavors in faba bean ingredients and meat alternatives, resulting in desirable sensory properties. Therefore, we tested the hypothesis by (1) investigating the sensory profile of the dextran-enriched faba bean protein concentrate (FPC) and extrudates containing 50% (dry matter, dm) of the dextran-enriched FPC, (2) determining the chemical characteristics of the FPC and extrudates, and (3) analyzing the relation between sensory descriptive data and chemical data.

2. Materials and methods

2.1. Materials

Faba bean protein concentrate was purchased from Suomen Viljava Oy (Helsinki, Finland), and contained 63.9% (dm) protein, 8% starch, 3.2% fat, 6.4% ash, and 9.5% moisture. Vital wheat gluten was purchased from Lantmännen Agro Oy (Vantaa, Finland), and contained 86.7% (dm) protein, 10.6% starch, 5% fat, 0.67% ash, and 6.5% moisture. Detailed descriptions of the methods and results for proximate analysis on raw materials are provided in Supplementary Material Table S1.

2.2. Production of dextransucrase and enzyme activity assay

The bacterial strain *W. confusa* A16 isolated from a traditional fried sourdough in Burkina Faso (Wang et al., 2019) was used to produce dextransucrase. The strain was routinely cultivated in MRS broth (Neogen, Heywood, UK) at 30 °C for 24 h. For the preparation of enzyme, the strain was subcultured in MRS broth with 2% (w/v) sucrose for 24 h at 30 °C. The overnight incubated culture (900 μL) was transferred to 45 mL of enzyme production medium (EPM, contained (g/L) sucrose, 20; maltose, 10; yeast extract, 20; dipotassium phosphate, 20; $MgSO_4{\cdot}7H_2O$, 0.2; $CaCl_2$, 0.01; NaCl, 0.01; $MnSO_4{\cdot}4H_2O$, 0.01; $FeSO_4{\cdot}7H_2O$, 0.01; and the pH was adjusted to 6.9 with 37% HCl) and incubated at 25 °C for 16 h. The total volume (45 mL) of EPM was then centrifuged (10 000 *g*, 4 °C, 15 min) to isolate the microbial cells, which were inoculated again in 450 mL of EPM at 25 °C for 6 h. The supernatant (cell-free extract) containing dextransucrase was obtained by centrifugation (10 000 *g*, 4 °C, 15 min) and stored at 4 °C until further use or analysis.

The enzyme activity was determined according to previous studies (Purama & Goyal, 2008; Shukla et al., 2014) with modifications. The assay of dextransucrase was carried out in a 1 mL reaction mixture containing 900 μL buffer solution (20 mmol/L sodium acetate buffer (pH 5.4), 5% sucrose, 0.3 mmol/L $CaCl_2$) and 100 μL cell-free enzyme extract. The reaction mixture was subsequently incubated at 35 °C for 30 min. The reaction was stopped by removing the enzymes using an Amicon Ultra-0.5 Centrifugal Filter Unit (10 kDa cut-off, Merck Millipore Ltd, Cork, Ireland), and centrifuged at 10 000 *g* for 10 min. The released fructose was analyzed using a high performance liquid chromatographic (HPLC) system as described in section 2.7.1. One unit of dextransucrase activity in the cell-free extract (U/mL) was expressed as the amount of enzyme to liberate 1 μmol of fructose per min of incubation time (Purama & Goyal, 2008). The dextransucrase activity was

followed at day 0, 1, 3, 6, 14 and 20 to study the storage stability (4 °C).

2.3. In vitro synthesis of dextran

Dextran was produced in a water solution using the enzyme extract. The incubation conditions were optimized to increase dextran yield, i.e., via different incubation times (24−48 h) and enzyme dosages (10%–20% v/v) (Supplementary Material Table S2). The optimal condition selected for dextran synthesis was 15% (v/v) cell-free extract in a distilled water solution that contained 10% (w/v) sucrose and 0.3 mmol/L $CaCl_2$ and was incubated at 35 °C for 24 h. The dextran-water solution was kept at 4 °C and used within two weeks.

2.4. Fermentative production of dextran in faba bean protein concentrate

For *in situ* production of dextran, *W. confusa* A16 was first cultivated in MRS broth anaerobically for 24 h at 30 °C. The cell cultures were obtained by centrifugation (9600 *g*, 10 min), washed with 0.01 mol/L sterile phosphate-buffered saline (PBS, pH 7.4, Merck, Steinheim, Germany), and resuspended in distilled water for inoculation.

Three types of FPC sourdough (dough yield 380, = (weight of sourdough/weight of flour in sourdough)*100) were prepared according to the recipe reported in Table 1. Three different preparations were made: (1) 24h Dextran-POS, where 10% flour weight (fw) of the FPC was replaced by sucrose (substrate for dextran synthesis) and fermentation was carried out at 25 °C for 24 h; (2) 11.4h Dextran-POS, where 10% (fw) of the FPC was replaced by sucrose and fermentation was at 23 °C for 11.4 h; and (3) 11.4h Dextran-NEG, where no sucrose was added and fermentation was at 23 °C for 11.4 h. For type 1 sourdough, the strain was inoculated at an initial cell density of ca. 6 log cfu/g, while for type 2 and 3 sourdoughs, the initial inoculation was ca. 7 log cfu/g. Of note, the fermentation conditions of type 2 sourdough were optimized (see Tuccillo, Wang, et al., 2022 for more detailed information) to maximize dextran production and minimize acid formation, since preliminary sensory trials revealed an association between intensive acidification and a strong and unpleasant sour smell and taste (Supplementary Material Fig. S1). The microbial growth including LAB, *Enterobacteriaceae*, and *B. cereus* in sourdough samples before and after fermentation were analyzed as previously reported (Wang, Xie, et al., 2022).

2.5. High moisture extrusion processing

Four types of extrudates – control, 11.4h Dextran-POS, 11.4h Dextran-NEG, and enzymatic dextran – were prepared according to Table 1 using a lab-scale twin-screw extruder (Process 11 Parallel twin-screw extruder, Thermo Scientific, Germany) equipped with a long cooling die (H × W × L, 5 × 20 × 250 mm). The screws were co-rotating with a diameter of 11 mm (L/D 40:1). During the extrusion, barrel temperatures were set as 145/150/150/145/110/80/70/60 °C, water feed was set as 300 mL/h, flour feed was 300 g/h, screw speed was 300 rpm, and the temperature of the cooling die was 40 °C. For all extrudates, the flour feed contained FPC and wheat gluten in a ratio of 50:50. Wheat gluten was added to promote the fibrous meat-like structure formation.

The samples “11.4h Dextran-POS extrudate” and “11.4h Dextran-NEG extrudate” were prepared by firstly freeze-drying the type 2 and 3 sourdoughs (section 2.4) with a Gamma 2–20 apparatus (Christ, Osterode a.H., Germany) and then rehydrating in distilled water (25% of total water content) at room temperature for 1 h prior to the extrusion process; this allows better solubility and functionality of the dextran. The water feed for these two samples was adjusted to 225 mL/h to keep a comparable moisture content (50%) as in the control extrudate (Table 1). The sample “enzymatic dextran extrudate” was prepared by replacing water feed with a 2% (w/v) dextran-water solution (section 2.3) resulting in 1% dextran (end-product basis) in the final extrudate. This concentration was selected due to the promising result (bitterness and pea flavor supressing effect) revealed in the preliminary sensory test (Supplementary Material Fig. S2).

The collected extrudates were vacuumed packed (Boss Verpackungsmaschinen GmbH & Co. KG, Germany) into polyamide-polyethylene pouches and stored at −20 °C prior to use. Approximately 30 g of the extrudates were freeze-dried and milled into powder

Table 1
Formulations of faba bean slurries and extrudates.

Slurries	Faba bean protein concentrate (g)	Water (mL)	Sucrose (g)	Initial cell density (log cfu/g)	Enzymatic dextran (4.3%, mL)	Lactic acid (85%, mL)	Acetic acid (10%, mL)
Control	150	420					
24h Dextran-POS [a]	135	420	15	6			
11.4h Dextran-POS	135	420	15	7			
11.4h Dextran-NEG	150	420		7			
Enzymatic dextran	150	262			165		
Chemically acidified	150	415				1.06	3.75
Acidified-dextran	150	257			165	1.06	3.75
Extrudates	Faba bean protein concentrate (g/h)	Water feed (mL/h)	Wheat gluten (g/h)	Freeze-dried sourdough (g/h)	Enzymatic dextran (2%, mL/h)	Water for hydration (mL/h)	
Control	150	300	150				
11.4h Dextran-POS		225	150	150		75	
11.4h Dextran-NEG		225	150	150		75	
Enzymatic dextran	150		150		300		

[a] 24h Dextran-POS, sample was fermented with *W. confusa* A16 at 25 °C for 24 h and the dextran yield was 5% (dm); 11.4h Dextran-POS, sample was fermented with *W. confusa* A16 at 23 °C for 11.4 h and the dextran yield was 4.73% (dm); 11.4h Dextran-NEG, sample was fermented with *W. confusa* A16 at 23 °C for 11.4 h with low dextran content (0.31% dm); Enzymatic dextran, sample contained 4.73% (dry matter) pure dextran produced by *W. confusa A16* dextransucrase; Chemically acidified, sample contained identical amount of lactic and acetic acids to 11.4h Dextran-POS; The dough yield was the same in all sourdoughs (380) and the moisture content was the same in all extrudates (50%).

(<300 μm) using a GRINDOMIX GM 200 (Retsch GmbH; Haan, Germany) for 40 s at 6000 rpm for further chemical analysis. The photos of the extrudates are presented in Supplementary Material Fig. S3.

2.6. Descriptive sensory analysis

2.6.1. Participants

Ten (six females and four males) volunteer study participants were recruited for evaluation of slurries, and thirteen (seven females and six males) for extrudates (Supplementary Material Methods). All volunteers were from the staff of the Department of Food and Nutrition at the University of Helsinki (UH) and had previous experience in sensory evaluation of faba bean-based products. Signed informed consent was obtained from all participants. Ethical principles of sensory study at the department were evaluated and approved by the University of Helsinki Ethical Review Board in the Humanities and Social and Behavioural Sciences (15/2020).

2.6.2. Preparation of slurry and extrudate samples

Six types of slurries were included in the sensory evaluation: (1) control slurry, prepared by mixing FPC and water using a blender (Oster, USA) for 60 s until completely homogeneous; (2) 24h Dextran-POS slurry, type 1 sourdough prepared in section 2.4; (3) 11.4h Dextran-POS slurry, type 2 sourdough prepared in section 2.4; (4) enzymatic dextran slurry, part of the water in the control slurry formulation was replaced by a dextran-water solution (prepared in section 2.3) to obtain a final dextran concentration comparable to that measured in the 11.4h Dextran-POS slurry; (5) chemically acidified slurry, part of the water in the control formulation was replaced by lactic and acetic acids in identical amounts to that measured in the 11.4h Dextran-POS slurry; and (6) acidified-dextran slurry, prepared with both dextran-water solution and lactic and acetic acids. For full recipes, see Table 1; for flowchart, see Fig. 1. All slurries had the same dough yield (380) and were prepared on the day of evaluation, except sample 3, which was prepared one day before evaluation and stored at 4 °C overnight. Four types of extrudates (prepared in section 2.5) were included in the sensory evaluation. The frozen extrudates were left at room temperature to thaw for 2 h prior to assessment. For the flow diagram, see Fig. 1. Slurries (ca. 20 g per sample) or extrudates (cut into equal lengths of 1.2 cm, 3 pieces per sample) were served at room temperature in plastic boxes covered with a lid to minimize moisture loss (see Supplementary Material Fig. S3) and were marked with random 3-digit codes.

2.6.3. Training and evaluation

The training and evaluation were carried out at the sensory laboratory (ISO8589) of UH. The participants took part in two 2-h training sessions to profile the sensory attributes of the slurries and extrudates, respectively. The sensory attributes, reference standards, method of evaluation, and evaluating order were developed by consensus during the training sessions as listed in Table 2. A total of 10 attributes (three odor, one texture, six taste/flavor) were selected for slurries, and 14 attributes (three odor, three texture, eight taste/flavor) for extrudates.

Three identical evaluations were performed on three independent days for slurries (June 2022) and extrudates (August 2022). Samples were presented in systematically randomized orders across the panelists and sessions under normal lightning conditions in individual sensory evaluation booths. The panelists were asked to rate the attributes on continuous visual analogue scales (0−10) while referring to the description and intensity of the reference standards when applicable (Table 2). Water and unflavored corn snacks were served for the panelists to cleanse their palate. The Fizz Acquisition 2.51 software (Biosystemes, Courternon, France) was used to collect the data.

2.7. Chemical analyses of slurries and extrudates

2.7.1. Determination of dextran

The dextran content in the sourdough samples (*in situ* synthesis) and dextran-water solutions (*in vitro* synthesis) were determined using an enzyme-assisted method with dextranase (Sigma-Aldrich, Germany) and α-glucosidase (Megazyme Ltd., Wicklow, Ireland), as previously reported (Katina et al., 2009). The analysis was done with an HPLC system that consists of an Alliance e2695 separation module (Waters, USA), a Waters 2465 pulsed amperometric detector and a waters 515 HPLC-pump for post column signal strengthening. The column used was CarbPac PA-1 analytical column (4 × 250 mm, Dionex Corporation, Sunnyvale, CA, USA). The separation was done with a gradient mode at a flow rate of 1 mL/min and column temperature of 30 °C with two eluents: Milli-Q water (eluent A) and 200 mmol/L NaOH (eluent B). A third eluent (eluent C, 300 mmol/L NaOH, flow rate 0.3 mL/min) was used for post-column addition. An internal standard 2-deoxy-D-galactose (Sigma-Aldrich, Schnelldorf, Germany) and a standard glucose (Merck, Darmstadt, Germany) were used for quantification.

2.7.2. Determination of titratable acidity, non-volatile flavor compounds and precursors

Five slurries (control, enzymatic dextran, 24h Dextran-POS, 11.4h Dextran-POS, and 11.4h Dextran-NEG) and four extrudates from the sensory evaluation were freeze-dried and analyzed for chemical profiles (Fig. 1). We note that the chemically acidified slurries were not included since the chemical composition was expected to be identical to that of the control slurry (the only difference was the acid addition). The pH and total titratable acidity (TTA) of slurries and extrudates were

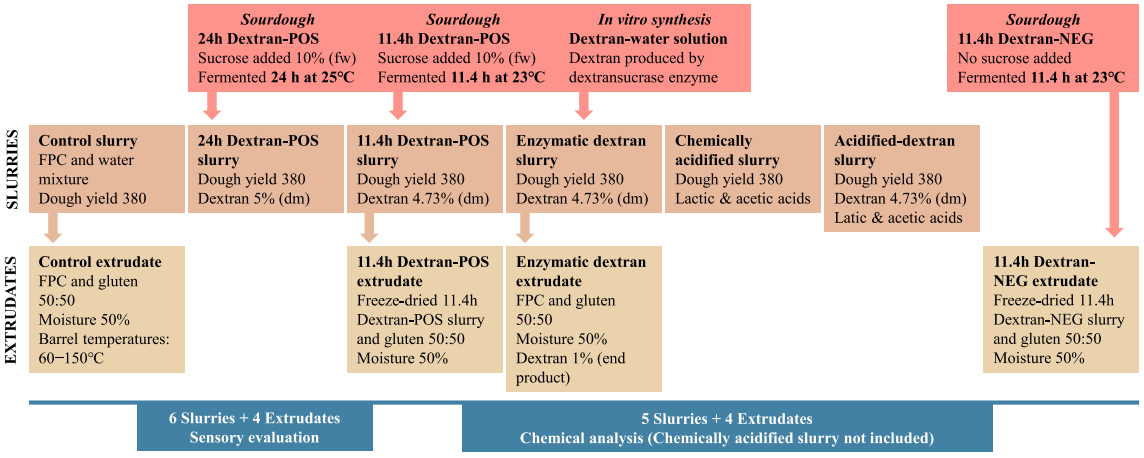

Fig. 1. Flowchart of slurry (including sourdough) preparation and types of extrudate made in this study.

Table 2
Lexicon, descriptions, reference standards, and their intensity used in the descriptive sensory analysis of slurries and extrudates.

Attribute	Description	Reference	Intensity
Slurries			
Sour odor	Sour odor of vinegar or sour milk products	Acetic acid (vinegar) 0.5% (v/v)	6
Pea odor	The odor of uncooked peas	Green peas overnight soaking in water and dried	6
Fruity odor	The odor of sweet, ripe fruits		
Runniness by spoon	How easily the sample flows out of spoon: bread dough (0) to water (10)		
Sweet taste	Typical sweet taste, like in table sugar	Sucrose 5% (w/v)	8
Bitter taste	Typical bitter taste e.g., coffee, beer, and some vegetables e.g., rucola	Caffeine 0.07% (w/v)	7
Sour taste	Typical sour taste, like in sour milk and pickled vegetables	Vinegar 0.5% (v/v)	8
Pea flavor	Flavor of uncooked peas	Green peas overnight soaking in water and dried	8
Astringency	Dry feeling in the mouth	Hand paper slices [a]	7
Overall aftertaste	Intensity of overall aftertaste (taste after swallowing/spitting the sample)		
Extrudates			
Layered appearance	Clarity of the layers when you break the sample from the middle		
Sour odor	Sour odor of vinegar or sour milk products	Vinegar 0.3% (v/v)	8
Cereal odor	Cereal, hay	Boiled oat hulls	4
Pea odor	The odor of cooked peas	Green peas overnight soaking in water and boiled	10
Fibrous texture	Separation into fibers, sinewy, muscle tissue		
Hard texture	The force required to bite sample, difficult to chew		
Sweet taste	Typical sweet taste, like in table sugar	Sucrose 3.5% (w/v)	8
Sour taste	Typical sour taste, like in sour milk and pickled vegetables	Vinegar 0.3% (v/v)	8
Bitter taste	Typical bitter taste, like in coffee, beer, and some vegetables like rucola	Caffeine 0.05% (w/v)	8
Umami taste	Meaty, savory, cooked protein products	Monosodium glutamate 0.25% (w/v)	10
Cereal flavor	Cereal, hay	Boiled oat hulls	6
Pea flavor	The flavor of cooked peas	Green peas overnight soaking in water and boiled	10
Astringency	Dry feeling in the mouth	Hand paper slices	8
Overall aftertaste	Intensity of overall aftertaste (taste after swallowing/spitting the sample)		

[a] Instructions for the use: Hold the piece of paper on top of the tongue for few seconds and take it away.

measured using an EasyPlus Easy pH Titrator (Mettler Toledo, Columbus, OH, USA). TTA was expressed as the volume (mL) of 0.1 mol/L NaOH used for adjusting the pH of a 10 g sample in 100 mL Milli-Q water to pH 8.5.

2.7.2.1. Free sugars, organic acids, free amino acids, and free fatty acids. Mono-, di- and oligo-saccharides, including galactose, glucose, sucrose, fructose, melibiose, raffinose, stachyose, and verbascose in slurries and extrudates (100 mg) were determined according to Xu, Wang, et al. (2017) using an HPLC system (see section 2.7.1). Organic acids were extracted from 500 mg of samples according to Wang, Zhou, and Liu (2020). The analysis was performed using an HPLC system with a Hi-Plex H column (300 × 6.5 mm; Agilent Technologies, USA), a Hi-Plex H guard column (50 × 7.7 mm; Agilent), and an ultraviolet detector (Waters 717, at 210 nm) and refractive index detector (HP 1047A, HP, USA). The mobile phase was 0.01 mol/L sulfuric acid (0.6 mL/min with 60 °C column temperature) and standards were L-lactic acid (Sigma-Aldrich) and acetic acid (Merck).

Free amino acids (FAAs) were extracted from 100 mg of samples by aqueous ethanol (70% v/v) as reported elsewhere (Wang, Natalia, et al., 2022). The derivatization of amino acids was accomplished by the AccQ•Tag Ultra kit (Waters, Milford, MA, USA). The separation was performed on a Waters BEH C18 column (2.1 × 100 mm, 1.7 μm particles, 55 °C column temperature) in an ultra-performance liquid chromatography (UHPLC) on an Acquity System (Waters). The eluents, gradient elution mode, and standards used were described in our earlier study (Wang, Natalia, et al., 2022). The chromatographic system used in this study was not able to separate glutamine and arginine, which were co-eluted and quantitated together by the standard curve of arginine. Free fatty acids (FFAs) were analyzed as described in Tuccillo, Kantanen, et al. (2022) with minor modifications. Samples of 1 g were extracted using accelerated solvent extraction (Dionex ASE-350; Thermo Fisher Scientific, USA) in triplicate. FFAs were analyzed using an HPLC (Waters Alliance 2690 Separations Module) with an Evaporative Light Scattering Detector (Waters 2420 ELSD, Waters; Milford, MA, USA) and separated on a LiChrosorb diol column (3 × 100 mm, 5 μm particles, VDS Optilab Chromatographie Technik GmbH; Berlin, Germany) using two solvents: 0.1% acetic acid in heptane (solvent A) and 0.1% acetic acid and 3% isopropanol in heptane (solvent B), at a flow rate of 0.5 mL/min and temperature of 25 °C. The gradient of solvents A and B was as follows: 0–5 min (97:3), 5–15 min (97:3 to 0:100), 15–35 min (0:100), 35–40 min (0:100 to 97:3), and 40–50 min (97:3). Free fatty acids were quantified using external standard calibration with oleic acid (Nu-Check-Prep; Elysian, MN USA) and separated as two peaks. The first consisted of palmitic and oleic acids, and the second of linoleic and α-linolenic acids.

2.7.2.2. Free phenolic content and profiles. Free (water-soluble) phenolic compounds were extracted from 100 mg samples by aqueous ethanol (80% v/v) according to Tuccillo et al. (2022). Free phenolic content was determined using the Folin–Ciocalteu (FC, Merck, Darmstadt, Germany) method (Singleton, Orthofer, & Lamuela-Raventós, 1999) with a UV spectrophotometer (UV1800, Shimadzu, Japan) at 765 nm. Gallic acid (Sigma-Aldrich, Steinheim, Germany) was used as a standard, and the phenolic content was expressed as mg gallic acid equivalents (GAE)/100 g freeze-dried sample. Phenolic profiles were analyzed using a validated ultra-high-pressure liquid chromatographic (UHPLC) method as described by Kylli et al. (2011). The Acquity Waters UPLC system (Bedford, MA, USA) was equipped with a binary solvent manager, sample manager, column manager, and photodiode array (PDA) detector. A Waters HSS T3 C18 column (2.1 × 150 mm, particle size 1.8 μm; Waters, Milford, MA, USA) was used to separate the free phenolic acids with linear gradient elution conducted with 0.5% formic acid in water and 0.5% formic acid in acetonitrile. The column temperature was kept at 40 °C and the constant flow rate of the mobile phase was 0.5 mL/min. The profiles of free phenolic acids were collected at 280 and 320 nm. Identification of the p-coumaric acids and ferulic acid was done based on their UV spectra and retention times of corresponding standards.

2.7.2.3. Condensed tannins, vicine and convicine. Condensed tannins were extracted from 100 mg samples with 1% H_2SO_4 (in MeOH, v/v) according to Wang, Natalia, et al. (2022) and measured using a UV

spectrophotometric method at 500 nm. Catechin (Sigma-Aldrich, Germany) was used as the standard and results were expressed as mg/100 g (dm) of catechin equivalent. Vicine and convicine were extracted from 100 mg samples using Milli-Q water (Tuccillo, Kantanen, et al., 2022) and analyzed using a Waters Acquity UPLC system (Bedford, MA, USA). The compounds were separated on a HSS T3 column (2.1 × 150 mm, 1.7 μm, C18 phase; Waters, Milford, MA, USA) at 25 °C. The elution was performed as an isocratic run for 6.5 min with MilliQ-water containing 0.1% formic acid at a flow rate of 0.35 mL/min. The column was washed with gradient elution; acetonitrile was raised to reach a proportion of 70% by 7.5 min and kept at that level until 9.5 min. The total run time with stabilization for the initial condition was 11 min. The data were collected at 273 nm. Vicine and convicine were identified based on their absorption maxima at 275 and 272 nm, respectively. In addition, vicine was confirmed according to its retention time. A six-point calibration curve at 273 nm was created for quantitation of vicine (Sigma-Aldrich, Germany), while convicine was semi-quantified from the standard curve of vicine.

2.7.3. Determination of volatile compounds

Volatile compounds of slurries and extrudates (fresh samples, 2 g) were analyzed using a headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) as reported in Lampi, Yang, Mustonen, and Piironen (2020). Samples were placed in 20-mL amber SPME vials and incubated for 10 min at 50 °C to reach equilibrium, followed by volatile compound extraction for 30 min at 50 °C using a divinylbenzenecarboxen-polydimethylsiloxane fibre (50/30 μm). The conditions were selected based on preliminary experiments to give repeatable peak area values of references (e.g., hexanal and hexanol). Volatile compounds were separated on a SPB-624 capillary column (30 m × 0.25 mm i.d. × 1.4 μm film thickness). Analysis of the volatiles was done by matching their MS spectra (total ion chromatograms from 40 to 300 *m/z*) against Wiley 7N databases (Wiley Registry™ of Mass Spectral Data) and by comparison with Linear Retention Indexes presented in Tuccillo, Wang, et al. (2022). Results were presented as peak areas (counts × s × 10^5) of the triplicate samples.

2.8. Statistical analysis

Sensory data and chemical data (three replicates) were analyzed using the SPSS Statistics 28.0 program (IBM SPSS Inc., Chicago, US) with one-way analysis of variance (ANOVA) followed by Tukey's post hoc test ($p < 0.05$). A two-way ANOVA was performed to check the panel performance by evaluating the significant levels of the main effects and two-way interactions (sample, panel, session, panel × sample, panel × session, and sample × session). Two separate partial least squares regression (PLSR) models were conducted to investigate the correlations between sensory attributes and chemical characteristics of the faba bean slurries and extrudates by using the R packages "FactoMineR" (Lê, Josse, & Husson, 2008) and "factoextra" (Kassambara & Mundt, 2019).

3. Results

3.1. Production of dextran in vitro and in situ, and microbial growth in sourdoughs

The cell-free extract of *W. confusa* A16 contained 7.9 U/mL crude dextransucrase enzyme and exhibited a good storage stability at 4 °C, with a slight decrease in activity (to 6.4 U/mL) after 20 days (Supplementary Material Table S3). The *in vitro* synthesis of dextran using the enzyme extract resulted in 4.34% (w/v) dextran in a water solution (Supplementary Material Table S2). The *in situ* production of dextran during fermentation of FPC with *W. confusa* A16 led to 5.04%, 4.73%, and 0.31% (dm) dextran in the 24h Dextran-POS, 11.4h Dextran-POS, and 11.4h Dextran-NEG sourdoughs, respectively (Table 3).

The initial cell density of LAB in 24h Dextran-POS sourdough was 6.1 log cfu/g, and increased by 3.5 log cycles after 24 h of fermentation (Table 3). The initial cell density of LAB in 11.4h Dextran-POS and 11.4h Dextran-NEG sourdoughs was ca. 7.0 log cfu/g, and increased by 2–2.2 log cycles after 11.4 h of fermentation. *Enterobacteriaceae* and *Bacillus* spp. were found in all sourdough samples at the beginning of fermentation at a cell density of approximately 3.3 and 2.6 log cfu/g, respectively. After fermentation, the cell densities of total *Enterobacteriaceae* and *Bacillus* spp. were significantly higher in 24h Dextran-POS sourdough (4.2 and 3.9 log cfu/g, respectively) than those of 11.4h Dextran-POS and 11.4h Dextran-NEG sourdoughs (3.5 and ca. 2.9 log cfu/g, respectively).

3.2. Acidity (pH and TTA) and organic acids of slurries and extrudates

The non-fermented control slurry had pH and TTA values of 6.2 and 7.8 mL, respectively (Table 4). The slurry enriched with *in vitro* produced dextran (enzymatic dextran slurry) had a similar pH, but slightly higher TTA (9.2 mL) than the control. The fermented slurries showed significantly lower pH and higher TTA compared to the control, with the highest acidity detected in 24h Dextran-POS sourdough (pH 4.7 and TTA 18.9 mL), followed by 11.4h Dextran-POS and 11.4h Dextran-NEG sourdoughs (pH 5.7 and TTA 11.4−11.7 mL). Correspondingly, a higher acidity level ($p < 0.05$) was found in extrudates prepared with 11.4h Dextran-POS and 11.4h Dextran-NEG sourdoughs (pH 6.1 and TTA 13.5−13.6 mL) compared to the control and enzymatic dextran enriched extrudates (pH 6.4 and TTA 10.3−10.9 mL).

In the control slurry and extrudate, organic acids were not detected (Table 4). A minor amount of lactic acid (161.0−194.9 mg/100 g dm) was found in the enzymatic dextran slurry and extrudate. In contrast, a high concentration of lactic acid (6014.1 mg/100 g) was observed in 24h Dextran-POS sourdough, which was 6-fold higher than that in 11.4h Dextran-POS and 11.4h Dextran-NEG sourdoughs. The concentrations of acetic acid (250.5−271.8 mg/100 g) were low in all sourdough samples. The extrudates made with 11.4h Dextran-POS and 11.4h Dextran-NEG sourdoughs showed comparable levels of lactic (867.1−913.3 mg/100 g) and acetic (155.6−195.8 mg/100 g) acids.

3.3. Non-volatile compounds

The sugar composition analysis revealed the existence of endogenous

Table 3
Microbial growth of faba bean slurries before and after fermentation and dextran production.

	Cell count 0 h (log cfu/g)			Cell count 11.4−24 h (log cfu/g)			Dextran (% dm)
	LAB	Enterobacteriaceae	*Bacillus* spp.[a]	LAB	Enterobacteriaceae	*Bacillus* spp.	
24h Dextran-POS	6.1 ± 0.2 [a]	3.3 ± 0.1 [a]	2.6 ± 0.1 [a]	9.6 ± 0.1 [c]	4.2 ± 0.2 [b]	3.9 ± 0.1 [b]	5.04 ± 0.25 [b]
11.4h Dextran-POS	7.0 ± 0.0 [b]	3.3 ± 0.2 [a]	2.4 ± 0.1 [a]	9.2 ± 0.1 [b]	3.5 ± 0.1 [a]	2.7 ± 0.2 [a]	4.73 ± 0.22 [b]
11.4h Dextran-NEG	6.9 ± 0.1 [b]	3.4 ± 0.2 [a]	2.6 ± 0.3 [a]	8.9 ± 0.1 [a]	3.5 ± 0.1 [a]	2.9 ± 0.1 [a]	0.31 ± 0.09 [a]

Different superscript letters in the same column indicate statistical significance ($p < 0.05$).
[a] Presumptive *Bacillus* spp. cell density, only white colonies detected.

Table 4
Condensed tannins, total free phenolics, free fatty acids, total free amino acids, vicine and convicine, organic acids, free sugars, and acidity (pH and TTA) of faba bean slurries and extrudates.

	Slurries					Extrudates			
	Control	Enzymatic dextran	24h Dextran-POS	11.4h Dextran-POS	11.4h Dextran-NEG	Control	Enzymatic dextran	11.4h Dextran-POS	11.4h Dextran-NEG
Galactose (% dm)	nd	nd	0.46 ± 0.01 ^{d}	0.36 ± 0.00 ^{b}	0.40 ± 0.01 ^{c}	nd	nd	0.10 ± 0.00 ^{a}	0.12 ± 0.01 ^{a}
Glucose (%)	0.03 ± 0.00 ^{ab}	0.03 ± 0.00 ^{ab}	0.02 ± 0.01 ^{a}	0.04 ± 0.01 ^{ab}	0.03 ± 0.01 ^{ab}	0.04 ± 0.01 ^{b}	0.04 ± 0.01 ^{b}	0.03 ± 0.01 ^{ab}	0.03 ± 0.01 ^{ab}
Fructose (%)	nd	4.29 ± 0.07 ^{e}	5.53 ± 0.05 ^{g}	5.32 ± 0.02 ^{f}	0.41 ± 0.02 ^{b}	nd	1.58 ± 0.05 ^{c}	1.90 ± 0.11 ^{d}	0.18 ± 0.02 ^{a}
Sucrose (%)	1.18 ± 0.03 ^{c}	1.16 ± 0.02 ^{c}	nd	0.06 ± 0.01 ^{a}	0.06 ± 0.01 ^{a}	0.63 ± 0.02 ^{b}	0.64 ± 0.04 ^{b}	0.06 ± 0.00 ^{a}	0.07 ± 0.02 ^{a}
Melibiose (%)	0.24 ± 0.02 ^{cd}	0.28 ± 0.02 ^{de}	0.29 ± 0.04 ^{de}	0.34 ± 0.04 ^{e}	0.27 ± 0.02 ^{de}	0.09 ± 0.03 ^{a}	0.12 ± 0.04 ^{ab}	0.16 ± 0.01 ^{ab}	0.18 ± 0.01 ^{bc}
Raffinose (%)	0.12 ± 0.02 ^{a}	0.12 ± 0.00 ^{a}	0.08 ± 0.04 ^{a}	0.09 ± 0.02 ^{a}	0.11 ± 0.05 ^{a}	0.12 ± 0.03 ^{a}	0.11 ± 0.01 ^{a}	0.09 ± 0.01 ^{a}	0.08 ± 0.02 ^{a}
Stachyose (%)	0.73 ± 0.01 ^{d}	0.76 ± 0.02 ^{d}	0.88 ± 0.06 ^{e}	0.93 ± 0.02 ^{e}	0.96 ± 0.02 ^{e}	0.33 ± 0.00 ^{a}	0.31 ± 0.02 ^{a}	0.45 ± 0.03 ^{b}	0.55 ± 0.03 ^{c}
Verbascose (%)	3.98 ± 0.00 ^{g}	3.80 ± 0.06 ^{f}	1.40 ± 0.05 ^{a}	2.73 ± 0.04 ^{d}	3.16 ± 0.06 ^{e}	2.35 ± 0.07 ^{c}	2.29 ± 0.11 ^{c}	1.41 ± 0.04 ^{a}	1.67 ± 0.01 ^{b}
Condensed tannins (mg/100 g dm)	112.51 ± 8.63 ^{e}	110.59 ± 9.56 ^{e}	72.24 ± 3.42 ^{d}	73.69 ± 2.21 ^{d}	82.61 ± 3.31 ^{d}	47.89 ± 4.40 ^{c}	47.40 ± 5.52 ^{bc}	35.74 ± 1.88 ^{a}	37.26 ± 2.94 ^{ab}
Total free phenolics (mg/100 g dm)	491.24 ± 3.34 ^{d}	491.68 ± 2.65 ^{d}	541.68 ± 5.36 ^{f}	511.15 ± 4.06 ^{de}	516.02 ± 9.42 ^{e}	233.69 ± 7.63 ^{a}	235.75 ± 7.96 ^{ab}	256.70 ± 8.03 ^{bc}	262.01 ± 12.01 ^{c}
Total free fatty acids (mg/100 g dm)	110.62 ± 3.76 ^{a}	131.46 ± 0.00 ^{b}	227.90 ± 5.26 ^{e}	177.76 ± 5.05 ^{c}	216.85 ± 10.11 ^{e}	188.23 ± 4.37 ^{cd}	195.53 ± 1.65 ^{d}	176.54 ± 2.14 ^{c}	195.01 ± 7.28 ^{d}
Free palmitic (16:0) & oleic (18:1) acids	89.51 ± 3.05 ^{bc}	82.20 ± 0.27 ^{ab}	111.54 ± 8.17 ^{d}	99.99 ± 3.38 ^{cd}	110.71 ± 1.39 ^{d}	71.90 ± 4.28 ^{a}	76.73 ± 3.19 ^{a}	75.34 ± 2.77 ^{a}	74.35 ± 7.19 ^{a}
Free linoleic (18:2) & α-Linolenic (18:3) acids	21.11 ± 0.72 ^{a}	49.26 ± 0.27 ^{b}	116.36 ± 3.17 ^{e}	77.77 ± 1.97 ^{c}	106.14 ± 9.45 ^{d}	116.34 ± 0.48 ^{e}	118.79 ± 1.62 ^{e}	101.20 ± 0.65 ^{d}	120.65 ± 1.72 ^{e}
Total free amino acids (mg/100 g dm)	1120.27 ± 59.71 ^{b}	1124.22 ± 15.96 ^{b}	2605.76 ± 133.00 ^{d}	1625.87 ± 251.56 ^{c}	1910.36 ± 113.48 ^{c}	586.05 ± 33.23 ^{a}	576.70 ± 65.96 ^{a}	888.98 ± 79.68 ^{b}	988.16 ± 53.22 ^{b}
Vicine (mg/100 g dm)	1105.60 ± 43.2 ^{d}	767.94 ± 59.40 ^{b}	782.84 ± 68.49 ^{b}	793.96 ± 57.15 ^{b}	971.79 ± 12.70 ^{c}	445.76 ± 9.35 ^{a}	410.86 ± 4.00 ^{a}	364.89 ± 18.01 ^{a}	447.60 ± 2.71 ^{a}
Convicine (mg/100 g dm)	789.46 ± 87.79 ^{c}	570.78 ± 75.33 ^{b}	634.71 ± 41.26 ^{bc}	603.11 ± 47.09 ^{b}	741.16 ± 7.62 ^{bc}	339.26 ± 8.59 ^{a}	316.72 ± 34.39 ^{a}	288.54 ± 60.37 ^{a}	347.36 ± 66.20 ^{a}
Lactic acid (mg/100 g dm)	nd	194.86 ± 1.30 ^{a}	6014.05 ± 250.76 ^{c}	961.60 ± 22.52 ^{b}	998.37 ± 46.48 ^{b}	nd	161.03 ± 5.17 ^{a}	913.33 ± 8.30 ^{b}	867.07 ± 22.59 ^{b}
Acetic acid (mg/100 g dm)	nd	nd	271.80 ± 45.80 ^{c}	250.51 ± 2.78 ^{bc}	302.96 ± 7.69 ^{c}	nd	nd	155.55 ± 3.44 ^{a}	195.81 ± 8.37 ^{ab}
pH	6.17 ± 0.06 ^{c}	6.05 ± 0.09 ^{c}	4.67 ± 0.01 ^{a}	5.65 ± 0.04 ^{b}	5.69 ± 0.01 ^{b}	6.51 ± 0.01 ^{d}	6.42 ± 0.02 ^{d}	6.08 ± 0.01 ^{c}	6.15 ± 0.01 ^{c}
TTA (mL)	7.80 ± 0.12 ^{a}	9.21 ± 0.11 ^{b}	18.86 ± 0.08 ^{g}	11.37 ± 0.16 ^{e}	11.65 ± 0.04 ^{e}	10.30 ± 0.07 ^{c}	10.85 ± 0.05 ^{d}	13.62 ± 0.05 ^{f}	13.52 ± 0.39 ^{f}

nd, not detected; Different superscript letters in the same row indicate statistical significance ($p < 0.05$).

sucrose (1.18% dm), glucose (0.03%), melibiose (0.24%), and raffinose family oligosaccharides, i.e., raffinose (0.12%), stachyose (0.73%) and verbascose (3.98%) in the control slurry (Table 4). The enzymatic dextran slurry exhibited a similar sugar profile as the control slurry with the exception of fructose, which was detected at a high level (4.3%). In the fermented slurries (i.e., sourdoughs), sucrose (endogenous and supplemented) was almost completely consumed. This resulted in ca. 5.5% fructose in 24h Dextran-POS and 11.4h Dextran-POS sourdoughs (with 10% sucrose addition) and 0.4% fructose in 11.4h Dextran-NEG sourdough (without sucrose addition). The fermented slurries exhibited a higher stachyose (ca. 0.9%) and a significantly lower verbascose (1.4%–3.2%) compared to the control; the highest reduction of verbascose was observed in 24h Dextran-POS sourdough. Additionally, galactose was found at ca. 0.4% in the fermented slurries. Correspondingly, the same trend was found in extrudates.

The amount of condensed tannins in the control and enzymatic dextran slurries was ca. 112 mg/100 g dm (Table 4). The fermented slurries had significantly lower levels of tannins (72−83 mg/100 g) compared to the control. Likewise, the extrudates prepared with fermented slurries (11.4h Dextran-POS and 11.4h Dextran-NEG sourdoughs) showed significantly less tannins than the control and enzymatic dextran extrudates. The total free phenolic content in the control and enzymatic dextran slurries was ca. 491 mg/100 g dm (Table 4). The fermented slurries showed a significantly higher content of total free phenolics (511−541 mg/100 g) than the control slurry, and the highest level was found in the 24h Dextran-POS sourdough. Similarly, the extrudates containing fermented slurries had higher free phenolic content ($p < 0.05$) compared to the control and enzymatic dextran extrudates. The chromatograms of phenolic profiles (data not shown) of the slurries and extrudates indicated no significant difference between groups (control, enzymatic dextran, and fermented samples). Only p-coumaric acid content was increased by 2–3 times after fermentation (data not shown).

The total free fatty acids (FFAs) content was 110.6 mg/100 g dm in the control slurry, which was composed of 81% palmitic (16:0) and oleic (18:1) acids and 19% linoleic (18:2) and α-linolenic (18:3) acids (Table 4). The enzymatic dextran slurry had slightly higher levels of FFAs (131.5 mg/100 g) than the control. The fermented slurries showed significantly higher levels of FFAs (177.8−227.9 mg/100 g) compared to the control slurry, and the FFAs composition was altered, consisting of 49%–56% palmitic and oleic acids and 44%–51% linoleic and α-linolenic acids. Linoleic acid was the most abundant FFA present in the fermented slurries, followed by oleic acid (Supplementary Material Fig. S4). The FFA content (176.5−195.5 mg/100 g) and composition were similar among extrudates, and the main FFA found was linoleic acid (Supplementary Material Fig. S4).

The total free amino acids (FAAs) content in the control slurry and enzymatic dextran slurry was comparable (ca. 1120 mg/100 g dm, Table 4). The fermented slurries showed significantly higher levels of FAAs (1626−2606 mg/100 g) than the control slurry, and the highest content was obtained in the 24h Dextran-POS sourdough. Similarly, the extrudates containing fermented slurries exhibited a significant increase in FAAs compared to the control. The effect of fermentation was also reflected in the amino acid compositions (Fig. 2). The concentrations of all the essential (histidine, lysine, threonine, methionine, isoleucine, leucine, phenylalanine, tryptophan, and valine) and non-essential amino acids (aspartic acid, glutamic acid, serine, glycine, alanine, proline, and tyrosine) in fermented slurries increased ($p < 0.05$) by up to 400 times compared to the control and enzymatic dextran slurries. The 24h Dextran-POS sourdough showed the highest amounts of all individual amino acids among all samples with the exception of co-eluted arginine/glutamine (the peak pair arginine/glutamine was overlapped). Arginine/glutamine and glutamic acid were the most abundant FAAs in all samples. Tryptophan was only found in detectable amounts in the fermented slurries. A similar trend in amino acid profiles was observed in extrudates.

The control slurry contained the highest amounts of vicine and convicine (1105.6 and 789.5 mg/100 g dm, respectively), followed by the 11.4h Dextran-NEG sourdough (Table 4). Levels of vicine and convicine were similar in the enzymatic dextran slurry, 24h Dextran-POS sourdough and 11.4h Dextran-POS sourdough, which were all lower than the control ($p < 0.05$). No significant difference was detected in the vicine and convicine contents of extrudates.

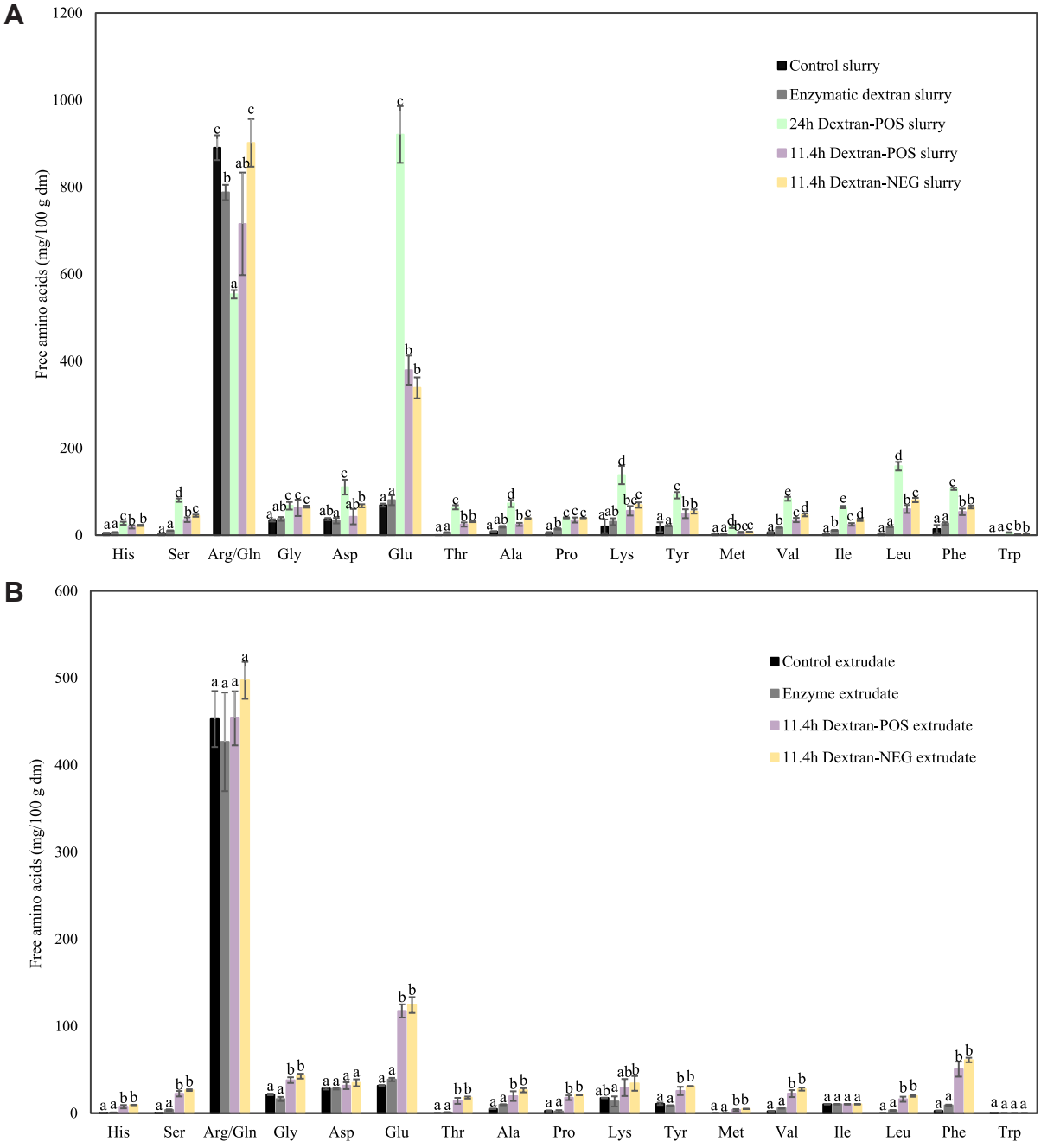

Fig. 2. Concentration of free amino acids (mg/100 g dm) of faba bean slurries (A) and extrudates (B). Error bars show standard deviation (n = 3). Different lowercase letters indicate significant differences ($P < 0.05$) within each amino acid.

3.4. Volatile compounds

Among the slurries and extrudates, 34 volatile compounds were detected, which belong to the following classes: organic acids, alcohols, aldehydes, alkanes, aromatic compounds, esters, ketones, and terpenes (Table 5). The majority of compounds consisted of alcohols and aldehydes, followed by alkanes and aromatic compounds. The lowest number of volatiles were detected in the control and enzymatic dextran slurries (12 and 10 compounds, respectively), followed by the fermented slurries (16 compounds) and extrudates (22−24 compounds). Acetic acid, isovaleric acid, ethyl acetate, and isoamyl isovalerate were detected in all sourdoughs, but not in the control and enzymatic dextran slurries. Several compounds were found in the extrudates, but not in the slurries, including 2- and 3-methyl-butanal, benzaldehyde, 2-ethylfuran, 2-butylfuran, 2-heptanone, octane, trimethyl-2,3,5-heptane, trimethyl-2,3,4-hexane, 3-methyl-decane, 5-methyl-undecane, and docosane. The opposite was observed for isobutyl alcohol, 3-hexen-1-ol, 2-hexenal, isoamyl isovalerate, hexanoic acid ethyl ester, 3-hydroxy-2-butanone, and 2,3-butanedione.

The control slurry had the highest levels of hexanal (peak areas of 257×10^5), whereas the lowest levels were observed in the 24h Dextran-POS, 11.4h Dextran-NEG, and 11.4h Dextran-POS sourdoughs (8×10^5, 16×10^5, and 35×10^5, respectively). Increased levels of hexanal were observed after extrusion for the 11.4h Dextran-NEG and 11.4h Dextran-POS sourdoughs, but the opposite was found for the control and enzymatic dextran slurries. 1-hexanol was the second most present compound in the control slurry (50×10^5). Significantly higher levels of 1-hexanol were observed after fermentation of the control slurry; these decreased after extrusion of the 11.4h Dextran-NEG and 11.4h Dextran-POS sourdoughs. Among all samples, the 24h Dextran-POS, 11.4h Dextran-NEG, and 11.4h Dextran-POS sourdoughs had the highest levels of 1-hexanol, ethanol, and ethyl acetate. Moreover, the 24h Dextran-POS sourdough had the highest levels of acetic acid, isovaleric acids, and 2,3-butanedione. Levels of acetic and isovaleric acids decreased after extrusion of 11.4h Dextran-NEG and 11.4h Dextran-POS slurries. The compounds that highly characterize the volatile profile of the extrudates were 2-pentylfuran and hexanal. The behavior of hexanal during extrusion was sample dependent, whereas for 2-pentylfuran, a significant increase during extrusion was observed for all samples.

3.5. Sensory profiles

Panel performance was assessed by investigating two-way interactions among sample, session, and panelist. The sample × session interaction revealed that the panel generated similar values ($p > 0.05$) across sessions in all attributes. The panelist × session interaction showed that panelists used the intensity scale across sessions, and in 10/11 attributes in slurries (significance in pea odor) and 13/14 attributes in extrudates (significance in cereal flavor), in a similar way ($p > 0.05$). The sample × panelist interaction showed similarities ($p > 0.05$) in the positioning on the intensity scale in none of the attributes for slurries and 2/14 attributes for extrudates. This can be associated with the genetic variation (taste receptor genes) and biological variation (saliva chemistry and taste thresholds) among individuals.

The sensory profiles of slurries are depicted in Table 6 The control slurry was characterized by high ratings of runniness, pea odor and flavor, bitter taste, astringency, and aftertaste. The chemically acidified slurry was assessed to be similar in sensory attributes to the control, with the exception of stronger sour taste, less pea odor, and lower runniness in texture ($p < 0.05$). The enzymatic dextran slurry differed significantly from the control, showing dramatically reduced scores for runniness, pea odor and flavor, bitter taste, astringency and aftertaste, while markedly higher ratings for sweetness ($p < 0.05$). The acidified-dextran slurry was identical in sensory characteristics to the enzymatic dextran slurry, expect for a significantly lower runniness. The 24h Dextran-POS slurry showed the highest ratings for sour odor and taste, fruity odor and aftertaste, while the lowest ratings for runniness and pea odor and flavor among all samples. Moreover, the bitterness was significantly reduced in the 24h Dextran-POS slurry compared to the control. The 11.4h Dextran-POS slurry exhibited a ca. 50% reduction in sour odor and flavor compared to 24h Dextran-POS slurry. The 11.4h Dextran-POS slurry was not significantly different from the enzymatic dextran and acidified-dextran slurries in any assessed attribute, expect sourness.

The sensory profiles of extrudates are reported in Table 7. The enzymatic dextran extrudate had a similar texture to the control extrudate, but was rated significantly lower in pea odor, bitter taste, and astringency, and higher in sweet taste and umami taste ($p < 0.05$). The 11.4h Dextran-NEG extrudate showed significantly higher scores in layered appearance and hard texture compared to the control extrudate, and was perceived to be significantly stronger in sour odor and taste, bitter taste, astringency, and aftertaste. The 11.4h Dextran-POS extrudate had a more layered appearance but hardness was comparable to the control. The 11.4h Dextran-POS extrudate was identical to the enzymatic dextran extrudate regarding all evaluated attributes ($p < 0.05$).

3.6. Correlations between chemical parameters and flavor properties

Partial least squares regression (PLSR) with 81 (or 83) chemical and flavor parameters was performed to assess how the parameters differ between the slurries (or extrudates) and how the chemical parameters were associated with the flavor properties (Fig. 3,4).

Regarding the PLSR of slurries, 98% of the variation in the chemical data explained 86% of the variation in the sensory data (Fig. 3). The control slurry was located negatively along both Component 1 and 2, and was associated with pea odor and flavor, bitter taste and astringency. The pea odor and flavor had strong positive correlations with nonanal and hexanal, and to a lesser extent, pentanal, heptanal, and 2-hexenal. The bitter taste and astringency were highly positively correlated to vicine and convicine, and to a less degree condensed tannins. The enzymatic dextran slurry was located negatively along Component 1 but positively along Component 2, and was characterized by sweet taste. Sweet taste had a correlation with glucose, melibiose and fructose. The 11.4h Dextran-POS slurry was located positively along Component 2 and close to the Y-axis, and was characterized by sweet taste and fruity odor. The fruity odor had strong positive correlations with isoamyl isovalerate and ethyl acetate. The 24h Dextran-POS slurry was located positively along Component 1 but negatively along Component 2, and was characterized by sour odor and taste and aftertaste. The sour odor had positive correlations with volatile compounds acetic acid and isovaleric acid, whereas sour taste was positively correlated with TTA, lactic and acetic acids, free phenolics, free palmitic and oleic acids, and free amino acids. Furthermore, a strong negative correlation was found between dextran and pea and bitter notes.

Regarding the PLSR of extrudates, 98% of the variation in the chemical data explained 96% of the variation in the sensory data (Fig. 4). The control extrudate was located negatively along Component 1 but positively along Component 2, and was closely associated with most of the evaluated flavor attributes e.g., pea odor and flavor, cereal odor and flavor, bitterness and astringency, and aftertaste. Pea odor and flavor had strong correlations with hexanal and heptanal. Cereal odor and flavor were highly correlated with 2-butylfuran. Bitter taste and astringency were related to vicine, convicine and arginine/glutamine. The enzymatic dextran extrudate was located positively along Component 1 and close to the X-axis, and was characterized by sweet and umami tastes. The sweet and umami tastes seemed to be linked to fructose and free palmitic and oleic acids. The 11.4h Dextran-POS extrudate was located negatively along both Component 1 and 2 and was associated with sweet, umami, and sour tastes. The 11.4h Dextran-NEG extrudate was located negatively along Component 1 but positively along Component 2, and was closely linked to sour odor and taste, bitter taste and aftertaste. Sour odor seemed to be associated with volatile compounds, e.g., 2-pentylfuran, 3-methyl-butanal, and trimethyl-2,3,5-

Table 5
Total peak areas (10^5) of volatile compounds in faba bean slurries and extrudates.

	RT (min)	*Slurries*					*Extrudates*			
		Control	Enzymatic Dextran	11.4h Dextran-POS	11.4h Dextran-NEG	24h Dextran-POS	Control	Enzymatic Dextran	11.4h Dextran-POS	11.4h Dextran-NEG
Organic Acids										
Acetic acid	8.722	nd	nd	17.3 ± 7.4 ^{a}	35.8 ± 5.9 ^{ab}	142.6 ± 21.4 ^{c}	0.6 ± 0.2 ^{a}	1.5 ± 0.2 ^{a}	72.2 ± 23.9 ^{b}	65.0 ± 10.1 ^{b}
Isovaleric acid	17.034	nd	nd	7.9 ± 1.6 ^{ab}	9.8 ± 0.3 ^{bc}	67.7 ± 3.2 ^{e}	2.7 ± 1.5 ^{a}	2.9 ± 1.2 ^{a}	15.9 ± 4.4 ^{cd}	16.9 ± 1.9 ^{d}
Alcohols										
Ethanol	3.569	nd	23.7 ± 3.4 ^{a}	62.6 ± 18.5 ^{b}	48.9 ± 4.1 ^{b}	211.5 ± 8.7 ^{c}	nd	22.0 ± 5.8 ^{a}	8.4 ± 2.3 ^{a}	12.2 ± 2.2 ^{a}
Isobutyl alcohol	4.519	nd	nd	2.5 ± 0.4 ^{a}	2.9 ± 0.3 ^{a}	nd	nd	nd	nd	nd
3-Methyl-1-butanol	11.754	2.0 ± 0.9 ^{ab}	1.9 ± 0.4 ^{ab}	3.7 ± 1.7 ^{bc}	4.6 ± 0.7 ^{c}	1.9 ± 0.1 ^{a}	nd	0.4 ± 0.1 ^{a}	nd	1.1 ± 0.5 ^{a}
1-Pentanol	12.954	3.0 ± 0.7 ^{ab}	2.0 ± 0.1 ^{a}	4.3 ± 0.3 ^{bc}	2.8 ± 0.1 ^{ab}	1.8 ± 0.3 ^{a}	4.9 ± 1.5 ^{c}	1.9 ± 0.5 ^{a}	1.7 ± 0.4 ^{a}	1.9 ± 0.5 ^{a}
1-Hexanol	16.665	49.5 ± 13.8 ^{ab}	83.0 ± 12.3 ^{bc}	236.8 ± 75.2 ^{e}	206.7 ± 17.1 ^{de}	154.6 ± 17.3 ^{cd}	6.5 ± 2.4 ^{ab}	3.4 ± 2.4 ^{a}	21.7 ± 3.9 ^{ab}	34.1 ± 3.8 ^{ab}
3-Hexen-1-ol	16.352	4.4 ± 0.4 ^{b}	8.6 ± 1.0 ^{c}	6.8 ± 1.7 ^{bc}	6.2 ± 0.5 ^{bc}	1.8 ± 0.2 ^{a}	nd	nd	nd	nd
Aldehydes										
2-Methyl-butanal	8.599	nd	nd	nd	nd	nd	1.2 ± 0.7 ^{a}	0.8 ± 0.3 ^{a}	nd	nd
3-Methyl-butanal	8.346	nd	nd	nd	nd	nd	2.2 ± 1.2 ^{a}	2.0 ± 1.1 ^{a}	7.7 ± 3.6 ^{b}	5.7 ± 1.1 ^{ab}
Pentanal	9.842	1.5 ± 0.4 ^{a}	nd	nd	nd	nd	6.7 ± 1.9 ^{b}	3.5 ± 0.4 ^{a}	2.5 ± 0.3 ^{a}	3.9 ± 0.2 ^{a}
Heptanal	17.510	1.6 ± 0.3 ^{a}	nd	nd	nd	nd	15.6 ± 3.9 ^{b}	12.8 ± 4.2 ^{b}	13.5 ± 3.0 ^{b}	15.4 ± 2.0 ^{b}
Hexanal	13.843	275.3 ± 54.4 ^{d}	112.9 ± 20.1 ^{c}	35.4 ± 5.5 ^{ab}	15.8 ± 3.8 ^{a}	7.6 ± 1.4 ^{a}	143.2 ± 35.7 ^{c}	89.6 ± 8.2 ^{bc}	98.0 ± 20.7 ^{bc}	113.9 ± 14.2 ^{c}
2-Hexenal	16.282	3.9 ± 0.5 ^{b}	3.2 ± 0.8 ^{b}	1.6 ± 0.4 ^{a}	0.7 ± 0.3 ^{a}	nd	nd	nd	nd	nd
Nonanal	24.522	3.3 ± 1.2 ^{a}	1.5 ± 0.3 ^{a}	0.5 ± 0.04 ^{a}	nd	nd	7.6 ± 2.0 ^{b}	7.2 ± 2.4 ^{b}	8.4 ± 0.4 ^{b}	7.7 ± 1.1 ^{b}
Alkanes										
Octane	12.139	nd	nd	nd	nd	nd	6.5 ± 2.3 ^{a}	3.9 ± 1.0 ^{a}	3.3 ± 0.5 ^{a}	3.9 ± 0.7 ^{a}
Trimethyl-2,3,5-heptane	19.628	nd	nd	nd	nd	nd	3.2 ± 1.2 ^{ab}	1.7 ± 0.2 ^{a}	3.5 ± 0.7 ^{ab}	4.0 ± 0.8 ^{b}
Trimethyl-2,3,4-hexane	19.451	nd	nd	nd	nd	nd	1.7 ± 0.6 ^{a}	1.2 ± 0.3 ^{a}	2.0 ± 0.7 ^{a}	1.9 ± 0.4 ^{a}
3-Methyl-decane	22.941	nd	nd	nd	nd	nd	7.2 ± 2.8 ^{a}	3.8 ± 0.6 ^{a}	10.5 ± 3.6 ^{a}	9.0 ± 2.4 ^{a}
5-Methyl-undecane	23.929	nd	nd	nd	nd	nd	16.2 ± 4.3 ^{ab}	10.4 ± 2.1 ^{a}	31.6 ± 9.8 ^{b}	28.4 ± 5.5 ^{b}
Docosane	31.698	nd	nd	nd	nd	nd	0.6 ± 0.1 ^{a}	0.5 ± 0.3 ^{a}	0.8 ± 0.2 ^{a}	0.6 ± 0.1 ^{a}
Aromatic Compounds										
Ethylbenzene	15.484	nd	nd	0.9 ± 0.2 ^{ab}	1.1 ± 0.2 ^{ab}	nd	7.5 ± 5.1 ^{b}	nd	nd	0.9 ± 0.1 ^{a}
Benzaldehyde	20.473	nd	nd	nd	nd	nd	nd	nd	9.3 ± 2.4 ^{a}	11.1 ± 0.3 ^{a}
2-Ethylfuran	9.205	nd	nd	nd	nd	nd	1.0 ± 0.8 ^{a}	nd	3.9 ± 0.6 ^{b}	4.5 ± 1.1 ^{b}
2-Butylfuran	16.298	nd	nd	nd	nd	nd	3.0 ± 0.8 ^{a}	1.8 ± 0.2 ^{a}	2.7 ± 0.3 ^{a}	2.8 ± 0.3 ^{a}
2-Pentylfuran	19.877	2.7 ± 1.1 ^{a}	0.7 ± 0.2 ^{a}	1.9 ± 0.6 ^{a}	2.7 ± 0.1 ^{a}	4.1 ± 0.3 ^{a}	172.1 ± 31.4 ^{bc}	143.2 ± 38.1 ^{b}	183.4 ± 20.5 ^{bc}	212.8 ± 14.3 ^{c}
Esters										
Ethyl acetate	6.810	nd	nd	78.8 ± 18.1 ^{b}	76.2 ± 6.1 ^{b}	128.2 ± 16.6 ^{c}	nd	nd	2.8 ± 1.3 ^{a}	6.4 ± 2.0 ^{a}
Isoamyl isovalerate	23.891	nd	nd	1.8 ± 0.3 ^{a}	4.0 ± 0.3 ^{b}	2.5 ± 0.3 ^{a}	nd	nd	nd	nd
Hexanoic acid ethyl ester	20.350	nd	nd	nd	nd	11.3 ± 1.1	nd	nd	nd	nd
Ketones										
3-Hydroxy-2-butanone	11.489	nd	nd	nd	nd	16.4 ± 2.2	nd	nd	nd	nd
2,3-Butanedione	6.406	nd	nd	nd	nd	161.7 ± 20.8	nd	nd	nd	nd
2-Heptanone	17.22	nd	nd	nd	nd	nd	4.8 ± 1.6 ^{a}	3.6 ± 1.7 ^{a}	4.9 ± 1.9 ^{a}	7.5 ± 1.0 ^{a}
Terpenes										
Delta-3-carene	20.423	8.4 ± 0.5 ^{bc}	4.0 ± 0.9 ^{a}	7.8 ± 1.5 ^{ab}	13.9 ± 0.9 ^{d}	12.1 ± 0.7 ^{cd}	13.5 ± 3.0 ^{d}	5.0 ± 1.0 ^{ab}	nd	nd
d-limonene	21.123	2.3 ± 0.7 ^{a}	nd	nd	1.7 ± 0.1 ^{a}	0.8 ± 0.1 ^{a}	5.8 ± 1.2 ^{b}	nd	nd	nd

nd, not detected; RT, retention time. Results are expressed as mean ± standard error (n = 3). Different superscript letters in the same row indicate statistical significance ($p < 0.05$).

Table 6
Sensory profiling of faba bean slurries.

Attributes of Slurries	Control	Chemically acidified	Enzymatic dextran	Acidified-dextran	24h Dextran-POS	11.4h Dextran-POS
Sour odor	0.7 ± 0.2 ^{a}	1.2 ± 0.2 ^{a}	1.3 ± 0.3 ^{a}	1.1 ± 0.3 ^{a}	6.2 ± 0.3 ^{c}	3.2 ± 0.4 ^{b}
Pea odor	5.3 ± 0.4 ^{c}	3.8 ± 0.3 ^{b}	3.7 ± 0.4 ^{b}	3.3 ± 0.4 ^{ab}	2.1 ± 0.3 ^{a}	2.7 ± 0.3 ^{ab}
Fruity odor	0.8 ± 0.2 ^{ab}	0.5 ± 0.1 ^{a}	1.1 ± 0.2 ^{abc}	0.8 ± 0.1 ^{ab}	1.7 ± 0.3 ^{c}	1.4 ± 0.3 ^{bc}
Runniness	9.1 ± 0.1 ^{d}	7.9 ± 0.2 ^{c}	2.9 ± 0.3 ^{b}	1.6 ± 0.2 ^{a}	1.2 ± 0.1 ^{a}	1.5 ± 0.2 ^{a}
Sweet taste	0.8 ± 0.2 ^{a}	0.9 ± 0.2 ^{a}	3.6 ± 0.3 ^{b}	3.3 ± 0.3 ^{b}	1.1 ± 0.3 ^{a}	2.6 ± 0.4 ^{b}
Bitter taste	6.6 ± 0.3 ^{b}	6.5 ± 0.3 ^{b}	3.3 ± 0.4 ^{a}	3.7 ± 0.4 ^{a}	3.6 ± 0.4 ^{a}	3.2 ± 0.4 ^{a}
Sour taste	0.9 ± 0.2 ^{a}	2.8 ± 0.4 ^{bc}	1.2 ± 0.3 ^{a}	2.0 ± 0.3 ^{ab}	7.5 ± 0.3 ^{d}	3.4 ± 0.5 ^{c}
Pea flavor	6.1 ± 0.4 ^{b}	4.5 ± 0.5 ^{ab}	4.2 ± 0.5 ^{a}	3.8 ± 0.4 ^{a}	2.9 ± 0.4 ^{a}	3.1 ± 0.4 ^{a}
Astringency	4.8 ± 0.5 ^{b}	4.9 ± 0.4 ^{b}	2.7 ± 0.4 ^{a}	3.1 ± 0.3 ^{a}	4.0 ± 0.4 ^{ab}	2.8 ± 0.3 ^{a}
Aftertaste	6.1 ± 0.4 ^{bc}	6.1 ± 0.4 ^{bc}	3.7 ± 0.4 ^{a}	4.6 ± 0.4 ^{ab}	6.9 ± 0.4 ^{c}	4.3 ± 0.4 ^{a}

Results are expressed as mean ± standard error (n = 3 replicates × 10 panelists). Different superscript letters in the same row indicate statistical significance ($p < 0.05$).

Table 7
Sensory profiling of faba bean extrudates.

Attributes of extrudates	Control	Enzymatic dextran	11.4h Dextran-POS	11.4h Dextran-NEG
Layered appearance	4.9 ± 0.3 ^{a}	5.2 ± 0.4 ^{ab}	6.3 ± 0.4 ^{bc}	6.6 ± 0.3 ^{c}
Sour odor	1.3 ± 0.3 ^{a}	1.2 ± 0.2 ^{a}	1.7 ± 0.3 ^{a}	2.9 ± 0.3 ^{b}
Cereal odor	4.5 ± 0.3 ^{ab}	3.7 ± 0.3 ^{ab}	3.6 ± 0.3 ^{a}	4.8 ± 0.4 ^{b}
Pea odor	3.4 ± 0.3 ^{b}	2.4 ± 0.3 ^{a}	2.0 ± 0.3 ^{a}	3.2 ± 0.3 ^{b}
Fibrous texture	4.8 ± 0.3 ^{a}	5.0 ± 0.4 ^{a}	4.5 ± 0.4 ^{a}	5.0 ± 0.3 ^{a}
Hard texture	4.0 ± 0.3 ^{a}	3.6 ± 0.3 ^{a}	3.9 ± 0.3 ^{a}	5.5 ± 0.3 ^{b}
Sweet taste	1.1 ± 0.1 ^{a}	2.5 ± 0.2 ^{b}	2.0 ± 0.2 ^{b}	0.7 ± 0.1 ^{a}
Sour taste	0.7 ± 0.1 ^{a}	0.9 ± 0.2 ^{ab}	1.8 ± 0.3 ^{b}	2.8 ± 0.4 ^{c}
Bitter taste	3.1 ± 0.3 ^{b}	1.8 ± 0.2 ^{a}	2.2 ± 0.3 ^{a}	4.5 ± 0.3 ^{c}
Umami taste	2.7 ± 0.3 ^{a}	3.9 ± 0.4 ^{b}	3.4 ± 0.3 ^{ab}	2.5 ± 0.3 ^{a}
Cereal flavor	4.1 ± 0.3 ^{a}	3.5 ± 0.3 ^{a}	3.8 ± 0.4 ^{a}	4.5 ± 0.5 ^{a}
Pea flavor	3.6 ± 0.4 ^{a}	2.3 ± 0.4 ^{a}	2.5 ± 0.3 ^{a}	3.4 ± 0.4 ^{a}
Astringency	3.3 ± 0.4 ^{b}	1.9 ± 0.2 ^{a}	2.4 ± 0.2 ^{ab}	4.5 ± 0.3 ^{c}
Overall aftertaste	4.1 ± 0.3 ^{a}	2.9 ± 0.3 ^{a}	3.5 ± 0.3 ^{a}	5.9 ± 0.4 ^{b}

Results are expressed as mean ± standard error (n = 3 replicates × 13 panelists). Different superscript letters in the same row indicate statistical significance ($p < 0.05$).

heptane, while sour taste was related to acetic and lactic acids, TTA, free phenolics, and free amino acids. Similar to the observations in slurries, dextran content in extrudates exhibited a strong negative correlation with pea odor and flavor.

4. Discussion

The global plant-based protein market is witnessing significant and rapid growth. However, off-flavors such as beany flavor and bitter taste are often present in plant-based proteins and products (Roland et al., 2017), hindering their adoption by a wider spectrum of consumers. To date, there is scant information on the reduction or masking of off-flavors – particularly bitterness – in plant proteins and their extruded meat alternatives (Wang, Tuccillo, et al., 2022). In the present study, we investigated whether dextran produced *in vitro* by enzymes or *in situ* during sourdough fermentation can mask off-notes and improve flavor properties of faba bean protein concentrate and extrudate.

The dextran yield *in vitro* by a dextransucrase enzyme isolated from *W. confusa* A16 was close to the theoretical amount (i.e., 5% from 10% added sucrose) allowed by this reaction. The dextran yield during fermentation of FPC with *W. confusa* A16 (24h Dextran-POS and 11.4h Dextran-POS sourdoughs) also approached the theoretical content (i.e., 5.6% from 11.2% added and endogenous sucrose). This confirmed the high efficiency of dextransucrase and dextran production by *Weissella* spp. (Shukla et al., 2014; Wang et al., 2022a, 2022c). The minor amount of dextran in 11.4h Dextran-NEG sourdough originated from the endogenous sucrose (1.2%) in FPC. The polymerization reaction of the glucose moiety of sucrose led to accumulation of fructose in the *in vitro* produced dextran solutions and fermented slurries.

Lactic acid was the dominant acid in all fermented slurries. Reducing the fermentation time from 24 to 11.4 h and temperature from 25 to 23 °C resulted in a substantial decrease in lactic acid formation and accordingly a lower level of acidity, which is consistent with previous studies (Tuccillo, Wang, et al., 2022). The minor amount of lactic acid in the enzymatic dextran slurry was attributed to the remnant acid from the crude enzyme extract. Furthermore, raw FPC can be contaminated with potentially harmful microbes, which growth should be prevented during the processing. Reducing the fermentation time seemed to improve the microbiological safety of the fermented slurries, considering lower and acceptable levels of *Enterobacteriaceae* (<4 log cfu/g) and *Bacillus* spp. (<3 log cfu/g) (EFSA BIOHAZ Panel, 2017).

By using isolated dextransucrase as the catalyst, dextran could be produced in high quantities and without any fermentation associated acid formation. The fermentation process, however, showed the advantages of reducing anti-nutritional factors (e.g., condensed tannins and raffinose family oligosaccharides (RFOs)) and improving free phenolic and free amino acid profiles of FPC and extrudates. Plant condensed tannins can be either free, bound to proteins, or largely bound to cellulose (Altop, 2019). The decrease of condensed tannins in FPC during fermentation might be due to the activation of residual endogenous and microbial enzymes such as tannases at low pH (Gobbetti, De Angelis, Di Cagno, Polo, & Rizzello, 2020). Verbascose was the major RFO in FPC, and its content decreased with increasing fermentation time, suggesting the activity of residual endogenous and/or microbial α-galactosidase (Xu et al., 2017a, 2017b). This was evidenced by the presence of galactose, i.e., the degradation product of verbascose, after fermentation (Xu et al., 2017a, 2017b). Furthermore, fermentation was reported to increase total and free phenolic content of legumes and enhance the antioxidant capacity (Gan, Shah, Wang, Lui, & Corke, 2016; Oboh, Ademiluyi, & Akindahunsi, 2009). The mechanisms involved in the modification of phenolics during fermentation might be (1) the activation of plant endogenous and/or bacterial enzymes, e.g., β-glucosidase, esterases, decarboxylases, reductases and hydrolases; (2) the release of phenolic compounds from there linked or conjugated forms (with proteins, sugars, or fatty acids); (3) the increased extractability; and (4) the metabolism of phenolic acids and synthesis of new compounds (Adebo & Gabriela Medina-Meza, 2020).

Fermented FPC exhibited higher FAAs content than the non-fermented ones, particularly considering the high amount of glutamic acid and essential amino acids (e.g., leucine and lysine). Apart from the

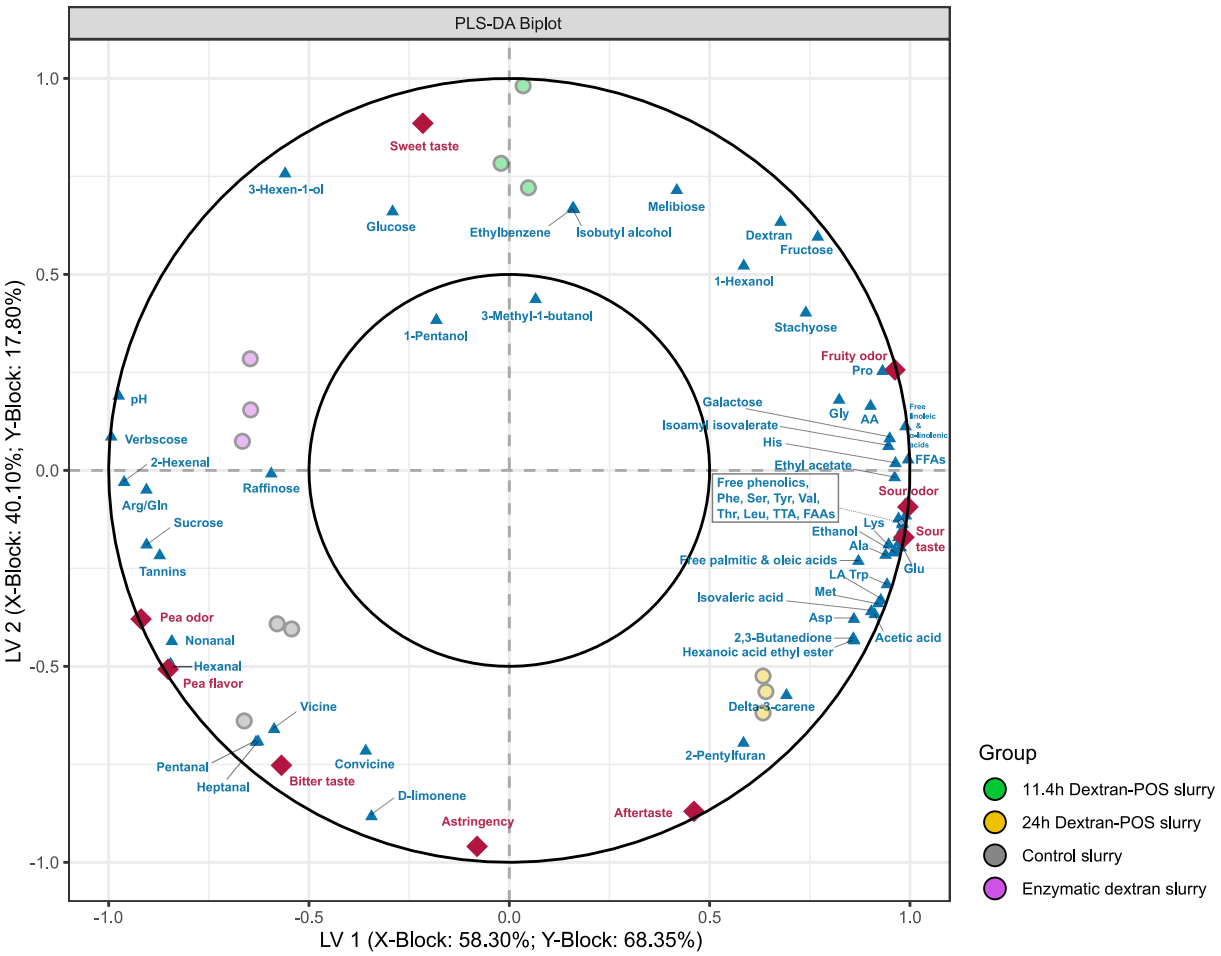

Fig. 3. Partial least squares regression (PLSR) biplot of the interaction between flavor related chemical parameters (predictors, triangles, in blue) and sensory properties (responses, squares, in red) of four FPC slurries (circles).

fact that FAAs can be absorbed rapidly and contribute to the nutrient need of the body, they impart taste or act as precursors for creating volatile flavor compounds in extruded plant-based meat through the Maillard reaction (Wang, Tuccillo, et al., 2022). The FAAs in fermented FPC are possibly generated by the degradation of proteins via endogenous and/or microbial proteolytic enzyme activities, e.g., proteases that release free amino acids (Christensen, García-Béjar, Bang-Berthelsen, & Hansen, 2022). Additionally, glutamine can be converted to glutamic acid by bacterial glutaminase activity and/or deamidation (a nonenzymatic hydrolytic reaction) that removes an amide functional group in the side chain (Vermeulen, Gänzle, & Vogel, 2007). Fermentation of FPC also changed the FFA profiles with increasing content of oleic and linoleic acids. The increased activity of lipolytic enzymes during fermentation possibly led to the production of free fatty acids (Adebo et al., 2022).

Fermentation of FPC or adding enzymatic dextran reduced the vicine content by up to 31%, whereas the convicine content was not affected. The use of fermented FPC in extrusion did not alter the vicine and convicine contents compared to the control extrudates. Vicine and convicine are stored in cotyledons of faba bean seeds (ca. 1% dm, Khazaei et al., 2019), and the industrial process for isolating proteins via dry milling and air classification concentrates the vicine and convicine in the protein fraction (ca. 2% dm in our study). Vicine and convicine are toxic (i.e., cause favism) in individuals with a genetically inherited deficiency in glucose-6-phosphate dehydrogenase (Khazaei et al., 2019). Further treatment with externally added β-glucosidase should be considered to achieve effective degradation of vicine and convicine (Khazaei et al., 2019; Pulkkinen et al., 2019).

The FPC slurries and extrudates were characterized by an intense pea odor and flavor, bitter taste, and astringency. The analysis of flavor-related chemical parameters and PLSR suggested that the presence of lipid-oxidation products such as hexanal, heptanal, and nonanal were related to the pea odor and flavor. FPC contains unsaturated fatty acids (mainly oleic and linoleic acids), which are oxidized through free radical chain reactions (auto-oxidation) and/or enzymatic pathways (endogenous lipoxygenases) to hydroperoxides that are further degraded in enzymatic or chemical reactions, generating volatile compounds that confer pea off-flavors (Wang, Tuccillo, et al., 2022). The bitterness and astringency of FPC were closely linked to the presence of vicine and convicine, which are not well-documented for their flavor properties in the literature (Nissim, Dagan-Wiener, & Niv, 2017; Wiener, Shudler, Levit, & Niv, 2012). Emerging evidence suggests that vicine and convicine contribute to bitter taste (Tuccillo, Kantanen, et al., 2022), although further research is needed for clarification. Additionally, condensed tannins, bitter tasting amino acids (e.g., arginine), and other secondary plant metabolites such as soyasaponin βg (Tuccillo, Kantanen, et al., 2022; Wang, Tuccillo, et al., 2022) may play a role in the perceived bitterness and dry mouthfeel of FPC and resulting products.

The addition of enzymatically produced dextran in FPC slurries and extrudates led to a significant decrease in pea, bitter, and astringent off-flavors, while also an appreciable increase in sweetness and umami taste (a specific taste for extrudate). The fructose liberated from sucrose by *W. confusa* dextransucrase activity seemed to be a main contributor to the sweet taste. The enhanced sweetness may help suppress off-notes in FPC (Keast & Breslin, 2003), but it did not appear to be a main factor. Our preliminary data indicated that fructose (8% dm) addition to FPC

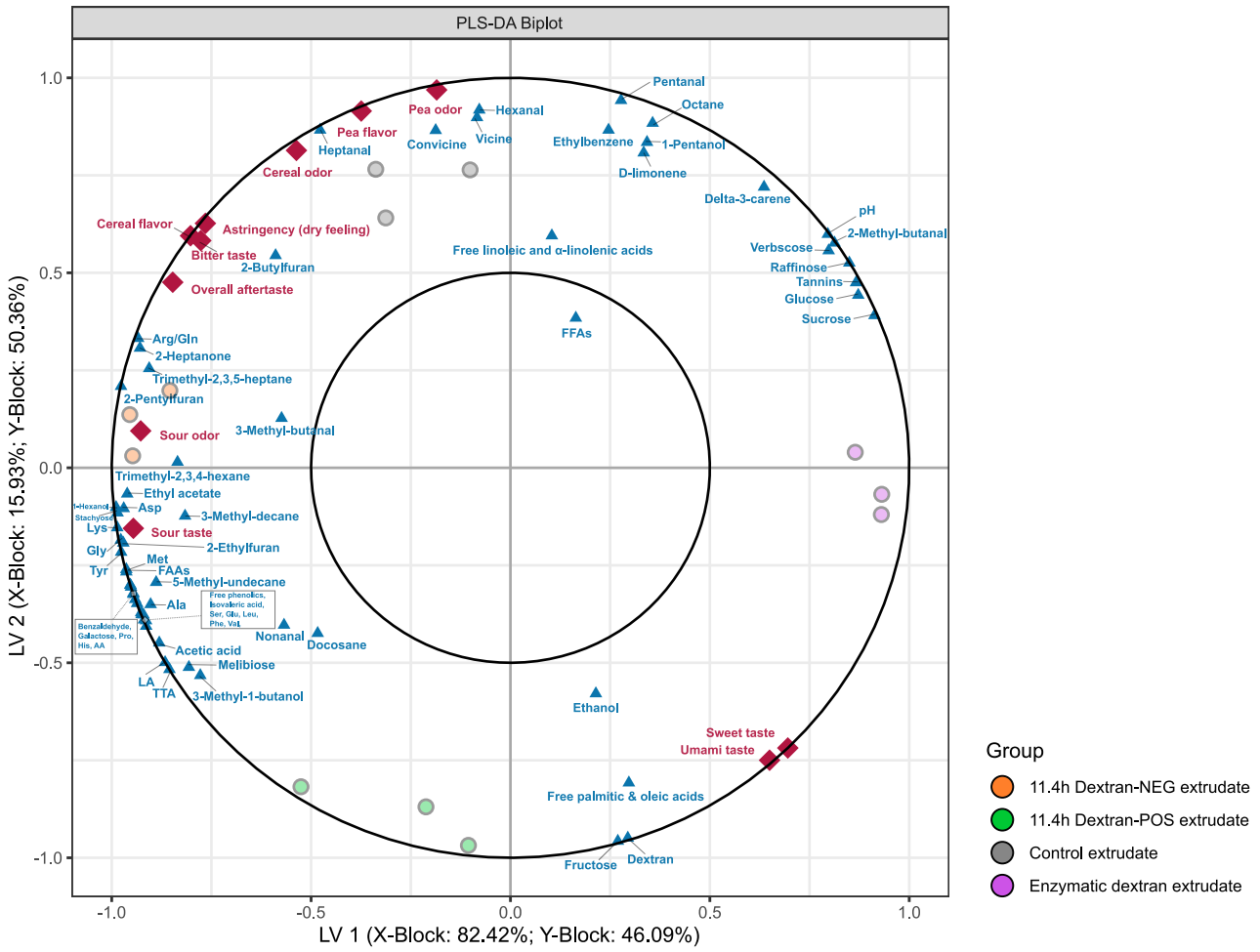

Fig. 4. Partial least squares regression (PLSR) biplot of the interaction between flavor related chemical parameters (predictors, triangles, in blue) and sensory properties (responses, squares, in red) of four FPC extrudates (circles).

did not significantly alter the bitterness, pea flavor, and aftertaste (Supplementary Material Fig. S5). The presence of dextran, which showed a negative correlation with pea and bitter notes, was the dominate factor contributing to off-flavor reduction in FPC slurries and extrudates. The enhanced umami taste is of great significance considering that a "meaty" flavor plays a crucial role in consumer acceptance of extruded plant-based meat (Fiorentini, Kinchla, & Nolden, 2020). The increased umami taste seemed to be unrelated to glutamic acid according to the PLSR model. Similar results were obtained in Valtonen et al. (2023), the extruded sausage prepared from fermented pea protein showed a more intensive umami taste compared to the non-fermented one, although no significant differences were found in glutamate content between the samples. Further studies are necessary in this respect to identify the presence of other potential umami tastants such as flavor nucleotides and peptides as well as the possible synergistic effect between the flavor substance on umami taste (Chen et al., 2021; Wang, Zhou, & Liu, 2020).

The inclusion of *in situ* synthesized dextran by fermentation in FPC slurries also showed promising results in masking pea and bitter off-notes. The reduced pea flavor might be partially attributed to the reduction of hexanal (a primary contributor to pea/beany notes) after fermentation, possibly due to microbial enzyme activities (e.g., alcohol dehydrogenase and aldehyde dehydrogenase) that converted hexanal into the corresponding alcohols or acids (Nedele, Gross, Rigling, & Zhang, 2021; Wang, Xie, et al., 2022). Nevertheless, the non-optimized fermentation conditions regarding the 24h Dextran-POS slurry led to formation of high levels of acids (e.g., acetic, isovaleric and lactic acids), and consequently an intense and undesirable sourness and aftertaste. In contrast, the optimized fermentation method concerning the 11.4h Dextran-POS slurry resulted in low levels of acids and a mild sourness and aftertaste. The sourness sensation may enhance or inhibit other taste qualities (Stevens, 1996). Our study suggested that the mild acidity, as evidenced by the chemically acidified slurry, had a suppressing effect on pea flavor, but not on bitter taste. Furthermore, the 11.4h Dextran-POS slurry was perceived as sweet and fruity, the latter of which was possibly due to the produced aroma-active esters such as isoamyl isovalerate and ethyl acetate during fermentation.

The optimized fermentation method (11.4h Dextran-POS slurry) functioned effectively in masking off-flavors and enhancing pleasant ones, e.g., sweet and umami tastes in extrudates. The use of 11.4h Dextran-NEG slurry (dextran negative control) in extrusion did not obtain the same positive effects, which instead intensified the sourness, bitterness, and aftertaste. Notably, the 11.4h Dextran-POS extrudate and the 11.4h Dextran-NEG extrudate had identical chemical profiles except for a different dextran content (1.2% and 0.1% fresh weight, respectively). On the other hand, the enzymatic dextran extrudate, which contained 1.0% (fresh weight) dextran, was evaluated to be similar in sensory profiles to the 11.4h Dextran-POS extrudate. Previous studies showed that the flavor masking effect of dextran was concentration dependent, i.e., a decrease in flavor perception with dextran presence at a concentration above its critical overlap concentration c* (0.43% of *W. confusa* A16 dextran, Wang, Trani, et al., 2020). Below the c* value, the presence of dextran did not affect flavor perception. Various mechanisms have been proposed to explain the hydrocolloid-flavor

interactions (Tournier, Sulmont-Rossé, & Guichard, 2007). In a liquid system, the polymer chains of hydrocolloids overlap and interpenetrate at or above c*, leading to a sharp increase in viscosity and a marked decrease in flavor perception. This was evidenced in our study by the less runny texture in the mouth and lower perceived flavor intensities in the enzymatic dextran and 11.4h Dextran-POS slurries (1.2% (fresh weight) dextran) compared to the control slurry. In a solid food matrix, a dextran concentration higher than c* may led to changed oral texture (e.g., cohesiveness and softness) and chewing behaviors, and consequently different flavor release kinetics (Wang, Trani, et al., 2020). Besides the physical entrapment (texture-specific effect), dextran may directly interact with flavor molecules via hydrogen bonding and other interactions (Tournier et al., 2007; Wang, Xie, et al., 2022).

5. Conclusion

The results from this work suggest that dextran produced *in situ* by fermentation with *W. confusa* A16, or *in vitro* via isolated dextransucrase enzyme is a promising flavor-masking agent for faba bean protein concentrate and extruded meat alternatives. Dextran was produced in substantial quantities that were effective in reducing pea flavor, bitter taste, and astringency. Pea flavor was related to hexanal, heptanal, and nonanal produced from the oxidation of unsaturated fatty acids, while bitterness and astringency were associated with vicine, convicine, and other bitter tasting compounds (e.g., tannins and bitter amino acids) inherent to faba beans. The optimization of fermentation conditions was crucial to minimize the undesirable sourness and aftertaste by reducing acid formation during the *in situ* production of dextran. Furthermore, the fructose and other flavor active compounds/precusors produced during the *in situ* or *in vitro* process contributed to pleasant flavors such as sweet and umami tastes of the extrudates. Notably, the *in situ* production of functional dextran by fermentation provides a dual advantage that delivers clean-label faba bean-based ingredients and meat alternatives with enhanced sensory and nutritional quality.

CRediT authorship contribution statement

Yaqin Wang: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Fabio Tuccillo:** Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Katariina Niklander:** Investigation, Methodology. **Greta Livi:** Formal analysis, Investigation, Methodology, Writing – original draft. **Aino Siitonen:** Investigation, Methodology. **Pinja Pöri:** Investigation, Methodology. **Minnamari Edelmann:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Hagrtoué Sawadogo-Lingani:** Writing – review & editing. **Ndegwa Henry Maina:** Writing – review & editing. **Kirsi Jouppila:** Writing – review & editing. **Anna-Maija Lampi:** Supervision, Writing – review & editing. **Mari Sandell:** Writing – review & editing. **Vieno Piironen:** Writing – review & editing. **Kaisu Honkapää:** Writing – review & editing. **Nesli Sözer:** Conceptualization, Supervision, Validation, Writing – review & editing. **Rossana Coda:** Conceptualization, Supervision, Validation, Writing – review & editing. **Antti Knaapila:** Conceptualization, Supervision, Validation, Writing – review & editing. **Kati Katina:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2023.109692.

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