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Predictive models for health-related and techno-functional properties

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Checklist	**Y (Yes), N (No)**
1) The deliverable has been sent to all relevant partners for review (i.e. in the same task/WP):	Y
2) The deliverable has been endorsed by the WP lead and co-lead:	Y
3) Input from other WPs has been sought, if applicable: ***Please specify:***	N

[1] **Type**: Use one of the following codes (in consistence with the Description of the Action):
R: Document, report (excluding the periodic and final reports)
DEM: Demonstrator, pilot, prototype, plan designs
DEC: Websites, patents filing, press & media actions, videos, etc.

[2] **Dissemination level**: Use one of the following codes (in consistence with the Description of the Action)
PU: Public, fully open, e.g. web
SEN: Sensitive, limited under conditions of the Grant Agreement

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Publishable Summary

This deliverable presents predictive models that link fermentation conditions to health-related, sensorial, and techno-functional properties of cereals and pulses and derived food products. Faba bean and oat were selected as representative plant-based ingredients due to their nutritional potential, but also their technological challenges, including beany off-flavours, low solubility, and the presence of anti-nutritional factors. To address these limitations, a series of liquid, semi-solid, and solid food systems were designed, including cereal-pulse beverages, oat-based emulsions, oat-faba gurts, tempeh-like products, extruded meat alternatives, and wheat breads enriched with sourdough from faba bean flour.

Across these models, fermentation conditions and processing parameters (e.g. microbial composition, inoculum ratio, time, temperature, pre-treatment, drying method) were varied. The resulting products were characterised for compositional, nutritional, and sensory properties, and the data were integrated using advanced statistical approaches such as principal component analysis (PCA), partial least squares (PLS) regression, principal component regression (PCR), and response surface methodology (RSM). These analyses provided insights into how fermentation parameters shape food quality across different matrices.

The models consistently demonstrated that fermentation strongly modifies product attributes. In faba bean ingredients, metabolomic profiling revealed links between specific compounds and sensory drivers of bitterness, astringency, and sweetness. In beverages, microbial consortia were key determinants of sensory clustering and consumer liking. In semi-solid gurts, RSM and PLS showed how inoculum ratio, time, and temperature drove acidity and viscosity development, which in turn influenced sensory perception. Tempeh-like products displayed enhanced proteolysis and nutrient accessibility during fermentation, while PLS modelling linked free amino acids, volatile compounds, and texture attributes to desirable sensory profiles. Extruded meat analogues highlighted the combined effect of fermentation and drying on flavour reduction (beany, bitter) and enhancement (umami, roasted). In breads, PCA revealed the contribution of sourdough fermentation to changes in protein and starch digestibility, with effects dependent on microbial composition. Finally, a joint PLS model integrating multiple food systems demonstrated that protein hydrolysis during digestion was most strongly associated with consortia-based fermentations, underlining the possible role of multi-strain systems in enhancing nutritional quality.

Together, these models establish clear relationships between fermentation conditions and the resulting nutritional, sensory, and techno-functional outcomes. The findings provide a framework for the rational design of plant-based foods, enabling more targeted use of fermentation to deliver products that are not only sustainable but also nutritious and appealing to consumers.

1. Introduction

The development of plant-based foods that are nutritious, palatable, and with favourable techno-functional properties is a central challenge in the transition towards sustainable diets. Cereals and legumes, such as oats and faba beans, are valuable raw materials due to their protein and fibre content as well as their lower environmental impact compared to animal-derived ingredients. However, their wider use in food products is hindered by undesirable flavours, poor functionality, and the presence of anti-nutritional factors that reduce nutritional quality.

Fermented foods are traditionally valued for their flavour, safety, and shelf stability, but the scientific evidence for their health benefits, beyond cultured dairy, remains limited.

Fermentation has long been used to improve the safety, shelf-life, and flavour of foods. Furthermore, fermented foods have gained a reputation for being beneficial to health. However, except for yoghurt and other cultured dairy products, little concrete evidence exists for the actual health benefits of

fermented foods. It has been shown that fermentation can reduce anti-nutrients, increase protein digestibility, and generate metabolites that influence taste, aroma, and texture. Yet, the effects of fermentation are highly dependent on raw material composition, microbial consortia, and process conditions such as fermentation time, temperature, and inoculum size. To optimise its application in modern food design, systematic approaches are required that can predictably link fermentation parameters to health-related, sensorial, and techno-functional properties.

This deliverable addresses this need by developing predictive models that link fermentation conditions to health-related, sensorial, and techno-functional properties of cereal–pulse raw materials and food systems. The models were applied across a diverse set of food systems, including liquid (cereal-pulse-based beverages and an oat-based drink model system), semi-solid (gurt from oat and faba bean), and solid systems (tempeh from oat and faba bean, extruded meat alternative, and wheat bread with faba bean sourdough). For each system, fermentation conditions were varied, and the resulting products were characterised for compositional, functional, nutritional, and/or sensory properties. Advanced statistical techniques, including principal component analysis (PCA), partial least squares regression (PLS), principal component regression (PCR), and response surface methodology (RSM), were applied.

By integrating chemical, sensory, and functional data across multiple food models, this deliverable demonstrates how predictive modelling can accelerate the design of novel plant-based products. The outcomes highlight the potential of fermentation not only as a traditional processing tool but as a science-guided strategy to deliver foods that meet consumer expectations for taste and texture while supporting nutritional quality and sustainability goals.

2. Description of Activities

2.1 Raw materials – faba bean ingredients

2.1.1 Experimental setup

Four commercially available faba bean ingredients from two manufacturers and two cultivar selections were investigated for chemical composition and sensory properties. Faba bean flour (FF) and faba bean protein concentrate b (FPCb) (cultivar Kontu) were obtained from Suomen Viljava Oy© (Helsinki, Finland), while faba bean protein isolate (FPI) and faba bean protein concentrate a (FPCa) (cultivars Snowbird, Tabasco, Malik, FBP-4) were sourced from AGT Food and Ingredients© (Regina, Canada). These ingredients were characterised sensorially and chemically using non-targeted metabolomic analysis.

The sensory profiles of the faba bean ingredients were generated using Generic Descriptive Analysis, which employed a trained panel (N = 10, 8 females) that was recruited from the Department of Food and Nutrition at the University of Helsinki based on prior experience with sensory profiling of similar plant-based samples. The entire study was conducted in a sensory laboratory following ISO 8589 standards. Panellists participated in three training sessions (1–2 h per session), during which they agreed on the lexicon, descriptions, and the type and intensity of reference samples. Training also covered the use of the scale, and panel performance was monitored using 3-way analysis of variance (ANOVA) and PCA. Samples were prepared as suspensions in tap water at a 1:3 (w/w) ratio, thoroughly mixed, and served (10 g) in plastic cups with lids labelled with three-digit codes at room temperature. Samples were presented in a randomised block design across sessions and assessors. Panellists were instructed to assess the intensity of each attribute by comparing the samples to a reference sample. Intensity was rated on a 0–10 line scale, with 0 representing “not at all” and 10 representing “very strong.” To cleanse their palate between samples, panellists drank water, chewed a puffed corn snack, and took a short break. Evaluations were conducted in triplicate under white light with controlled air conditioning. Data were collected using RedJade© software (RedJade Sensory Solutions LLC, Boulder, CO, USA).

For non-targeted metabolomic analysis, 100 mg of each sample was extracted with 80% cold methanol, centrifuged, diluted, filtered, and analysed in triplicate, with pooled quality control (QC) and blank samples included. Analyses were performed on a UHPLC-qTOF-MS system (Agilent Technologies) using both HILIC and reversed-phase (RP) chromatography and electrospray ionisation in positive and negative modes. The sample sequence was randomised with QC injections for system equilibration and ongoing performance checks. Data were processed using the notame analytical workflow and MS-DIAL software for peak detection, alignment, and filtering, resulting in 41,151 initial features. Quality (QF) and relevance filters (RF) were applied, including detection rate, blank subtraction, signal-to-noise ratio, MS/MS availability, fold change thresholds, ANOVA ($p < 0.01$), and Variable Importance in Projection (VIP > 1), reducing the dataset to 122 high-confidence features. Features were annotated semi-automatically using internal and public spectral libraries and further curated manually, with identifications classified from Level 1 (confirmed by standards) to Level 4 (unknowns).

2.1.2 Multivariate analysis

To associate the annotated features (predictors) with the sensory attributes (responses), a PLS model was generated using The Unscrambler® X (v. 10.5, Camo Software, Oslo, Norway). The model utilised the average peak areas of the annotated features across all samples, with both chemical and sensory datasets being auto-scaled and mean-centred. Samples were included as dummy variables, and the model was validated using full cross-validation and the kernel algorithm.

2.2 Liquid food systems – cereal-pulse-based beverages

2.2.1 Experimental setup

The aim of the study on cereal-pulse-based beverages was to evaluate the synergistic effect of microbial consortia and plant-based matrices (faba bean protein concentrate, faba bean starch concentrate, and oat flour) on the sensory properties and *in-vitro* protein digestibility of newly designed cereal-pulse-based beverages (CPBBs). A total of seven fermented CPBB samples were prepared, which differed in the microbial consortia used as starters (Figure 1). Binary, ternary, and quaternary consortia consisting of unconventional yoghurt starter cultures (*Lacticaseibacillus paracasei*, *Leuconostoc citreum, Levilactobacillus brevis*, and *Levilactobacillus namurensis*), which were selected based on their pro-technological (growth and acidification kinetics, and enzymatic activities), nutritional (peptides, free amino acids release, and anti-nutritional factor reduction), and functional (phenolic compounds release and exopolysaccharides (EPS) production) characteristics, were used. CPBBs were developed at a laboratory scale. The inclusion level of each ingredient was optimised through preliminary trials to define a baseline food product with satisfactory sensory characteristics. The final recipe included oat flour (5%, w/w), faba bean protein concentrate (4%, w/w), faba bean starch concentrate (1%, w/w), sugar (1.5%, w/w), salt (0.5%, w/w), calcium phosphate (0.5%, w/w), sunflower oil (3%, v/w), apple purée (10%, w/w), and tap water (74.5%, v/w). All ingredients were mixed adequately under continuous stirring for 5 min, followed by homogenisation with an Ultra-Turrax homogeniser (20,000 rpm for 1 min). The initial pH of the mixture was adjusted to 6.8 pH units with sodium hydroxide (2M), and then pasteurised at 90°C for 15 min using Thermomix Bimby® (Vorwerk, Italy). Prior to fermentation, CPBB was cooled down to ca. 35°C. Fermentation was performed with defined microbial consortia (CPBB-Started) at 30°C for 20 h. Two control CPBB samples were also included: CPBB-Raw, without bacterial inoculum and incubation, and CPBB-Unstarted, without bacterial inoculum but incubated under the same conditions as Started-CPBB. Sensory evaluation of the raw and fermented CPBB samples was carried out at the ISO 8589-compliant sensory laboratory at the University of Helsinki. Ninety-one volunteers were recruited through university mailing lists, social media, and word of mouth. CPBB samples (10–15 g) were presented at room temperature in transparent glass cups, covered and coded with random three-digit numbers.

Evaluations were performed under white light in individual booths using Compusense Cloud software (Guelph, Canada). A projective mapping (PM) combined with the check-all-that-apply (CATA) method was used to evaluate similarities and dissimilarities between sensory profiles of CPBB samples. Participants positioned samples, represented by icons, on the space provided by the software (650 x 650, white background) based on perceived similarity, and selected applicable sensory attributes from a predefined list (e.g., apple, beany, cereal, sour, umami, and other) or gave their own descriptors. After the mapping test, participants rated odour, taste, and overall liking on a 9-point hedonic scale. In addition, aliquots of raw and fermented CPBB were *in vitro* digested until the intestinal phase following the INFOGEST protocol (Brodkorb et al., 2019), after which the degree of protein hydrolysis was determined.

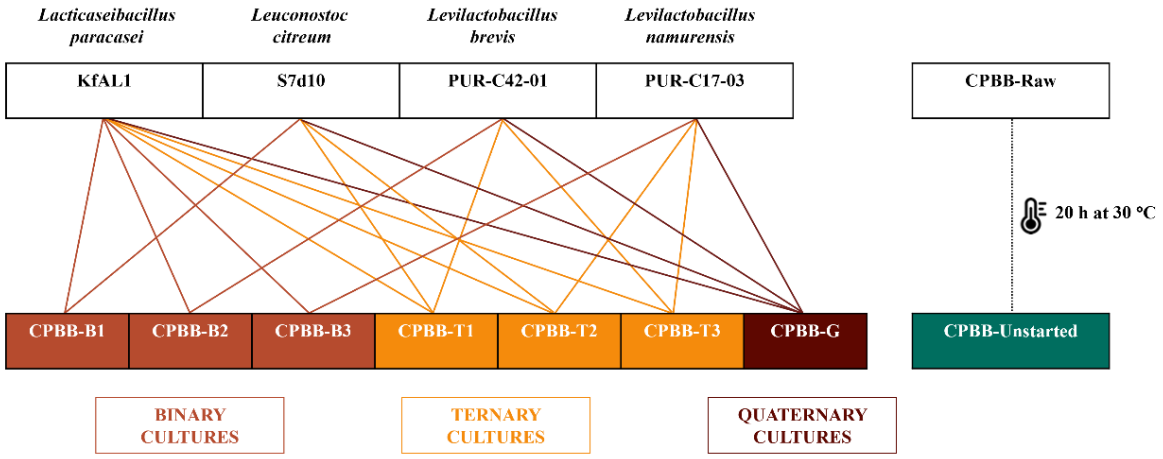

Figure 1. Schematic representation of the experimental design of mixed starter cultures for fermented cereal-pulse-based beverages.

2.2.2 Multivariate analysis

Principal Component Regression (PCR) was carried out to investigate the relationship between individual coordinates of CPBB samples (used as the X matrix, n = 182) and sensory descriptive data (used as the Y matrix, n = 46). The analysis was performed using the Singular Value Decomposition (SVD) algorithm, and a full cross-validation method was employed. PLS regression was then applied to predict liking of taste, odour, and overall liking (Y matrix, n = 3) from the sensory descriptors (X matrix, n = 46, with samples included as down-weighted dummy variables). The analysis was carried out using the Kernel algorithm, and a full cross-validation method was employed. The Unscrambler® X software (Version 10.5, Aspen Technology® Inc, Bedford, MA, USA) was used to conduct the analyses.

2.3 Liquid food systems – oat-based drink model system

2.3.1 Experimental setup

This experiment on an oat-based drink model system aimed to evaluate how fermentation affects various chemical and functional properties, and to identify the parameters that govern the stability of oat-based oil-in-water emulsions. Four treatment conditions were applied to both kilned and non-kilned oat wholemeal in duplicate, as outlined in Table 1. Two non-fermented oat wholemeal suspensions (1:6 ratio (w/v)) served as reference samples: one at the native pH (6.4) and the other adjusted with lactic acid to the pH value after fermentation (4.3 for kilned oat and 3.4 for non-kilned oat). For the third condition, fermentation was carried out by inoculating the suspensions (1:6 ratio (w/v)) with a commercial *L. plantarum* starter culture (Chr. Hansen) at a concentration of 10^7 CFU/g suspension, followed by incubation at 30 °C for 24 hours. The fourth treatment involved incubation with malted wheat extract (at 8.0 % (w/v)) in the presence of antibiotics to inhibit microbial growth.

The latter condition was included since preliminary experiments showed that the addition of wheat malt extract resulted in an increased protein extractability compared to fermented samples.

Table 1. Overview of the sample set used for preparing oil-in-water emulsions.

Sample	Raw material	Condition	Incubation time	pH value
1	Kilned oat	Reference	0 h	6.4
2	Kilned oat	Reference at low pH	0 h	4.3
3	Kilned oat	Fermented	24 h	4.3
4	Kilned oat	Incubated with wheat malt extract	24 h	4.3
5	Non-kilned oat	Reference	0 h	6.4
6	Non-kilned oat	Reference at low pH	0 h	3.4
7	Non-kilned oat	Fermented	24 h	3.4
8	Non-kilned oat	Incubated with wheat malt extract	24 h	3.4

Next, to gelatinise and hydrolyse the starch, α-amylase (50 U/g wholemeal) and amyloglucosidase (50 U/g wholemeal) were sequentially added to the treated kilned and non-kilned oat wholemeal suspensions, after which they were incubated respectively for both enzymes at 80 °C for 10 min, and 50 °C for 15 min, under continuous shaking at 150 rpm. After enzymatic treatment, the suspensions were centrifuged at 10,000 × g for 20 min. The resulting supernatants were immediately heat-treated in a boiling water bath for 10 min to inactivate all enzymes and then frozen for later analysis. These extracts were subsequently analysed for the parameters listed in

Table 2, using the corresponding analytical methods.

Table 2. Overview of the analysed chemical and functional properties of the extracts and emulsions, along with their corresponding analytical methods.

Extract property	Unit	Method
Protein extractability	% (w/w)	Total combustion method (Micro Dumas protein analysis system)
Average protein molecular weight	Da	Size-exclusion high-performance liquid chromatography
β-glucan extractability	% (w/w)	Colourimetric method (Megazyme)
Relative viscosity	-	Brookfield rheometer
ζ-potential	mV	Zetasizer Nano ZS
Emulsion property	**Unit**	**Method**
Average oil droplet size (24h)	µm	QICPIC dynamic particle sizer
Creaming index (24h)	-	Visual

Then, for O/W emulsion preparation, thawed extracts were supplemented with 2% (w/w) sunflower oil and pre-emulsified using an IKA Ultra-Turrax T18 digital homogeniser at 10,000 rpm for 5 min. Fine emulsions were obtained by ultra-high-

pressure homogenisation at 39–42 MPa using a Stansted Pressure Cell Homogeniser SPCH-EP at room temperature. These emulsions were characterised for their oil droplet size and creaming index (

Table 2), both 24 h after preparation.

2.3.2 Multivariate analysis

Multivariate analysis of chemical and functional properties in the extracts and O/W emulsions was performed in JMP Pro 17 (SAS Institute, Tervuren, Belgium). Pearson correlation coefficients were calculated between all variables, and a colour map of correlations was generated to visualise the relationships. Statistical significance of the correlations was assessed, and significant correlations ($p < 0.05$) were indicated with asterisks.

2.4 Semi-solid food systems – gurt from oat and faba bean

2.4.1 Experimental setup

The objective of the experiments described in this section was to gain insight into the use of oat and faba bean raw materials for developing a palatable, nutritious, and safe fermented semi-solid dairy alternative ('gurt').

First, a model system for a gurt was used to investigate the effect of fermentation conditions on pH, total titratable acidity, and viscosity by using Response Surface Methodology (RSM). The model system contained only flour ingredients and water: whole grain oat flour (WOF), having 14% protein and 11% fibre (Raisio Nutrition Ltd, Nokia, Finland), oat bran concentrate (OBC), having 23% protein and 37% fibre (Raisio Oyj, Raisio, Finland), faba bean flour (FF), having 30% dm protein and 12% dm fibre (Suomen Viljava Oy, Helsinki, Finland), faba bean protein isolate (FPI), having 90% dm protein and 3% fibre (AGT Food and Ingredients, Regina, Canada) and regular tap water. The composition by percentage of the mixture was 8.2 % WOF, 5.2 % OBC, 3.1 % FF, 2.07 % FPI, with 81.43 % of water. This recipe, with water included, was designed to achieve a protein and fibre content of the mixture of 5.2 % and 3.3 %, respectively. This mixture was fermented with a commercial yoghurt starter, consisting of *Bifidobacterium* sbsp., *Lactobacillus acidophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus paracasei* and *Streptococcus thermophilus species*. Experimental design is reported in Table 3. Time (7 to 18 h), temperature (30 to 45 °C), and inoculum ratio) 0.01 to 1% w/w) have been used as fermentation conditions. A Central Composite Face (CFF) was used as a model with 19 experiments, no replicates, and five centre points for assessing the reproducibility and reliability of the model.

In a next experiment, two types of gurt were produced: low-protein low-fibre control (LPLF), similar to a commercially available product, and high-protein high-fibre gurt (HPHF) containing high protein and fibre levels.

LPLF consisted of 8% wholegrain oat flour (WOF) and 88.2% water, while HPHF included 8% WOF, 5% oat bran concentrate (OBC), 3% faba bean flour (FF), 2% faba bean protein isolate (FPI), and 78.2% water. Both had salt (0.06%), sugar (3%), rapeseed oil (0.4%), calcium carbonate (0.09%), and tricalcium phosphate (0.27%).

The gurt-making process conducted in a Vorwerk Thermomix TM 6 blender cooker (Vorwerk & Co Thermomix GmbH, Wuppertal, Germany) involved blending the dry ingredients and oil in water for 2 minutes, heating the mixture to 60°C, and then adding an enzyme solution. LPLF was treated with α-amylase (3.36 µl/100 g) for 15 minutes, while HPHF was treated with a mixture of α-amylase, amyloglucosidase, and β-glucanase (3.36, 7, and 0.6 µl/100 g) for 1 hour, to monitor the viscosity affected by a higher amount of starch and β-glucan.

Table 3. The experimental design matrix includes the fermentation conditions of each experiment.

Experiment Number	Time (h)	Temperature (°C)	Inoculum Ratio (% w/w)
N1	7	30	0.01
N2	18	30	0.01
N3	7	45	0.01
N4	18	45	0.01
N5	7	30	1
N6	18	30	1
N7	7	45	1
N8	18	45	1
N9	7	37.5	0.1
N10	18	37.5	0.1
N11	12.5	30	0.1
N12	12.5	45	0.1
N13	12.5	37.5	0.01
N14	12.5	37.5	1
N15	12.5	37.5	0.1
N16	12.5	37.5	0.1
N17	12.5	37.5	0.1
N18	12.5	37.5	0.1
N19	12.5	37.5	0.1

After the enzymatic treatment, the mixtures were heat-treated at 85°C for 5 minutes and then cooled to 40°C. The mixture was divided into three bottles and a commercial starter, VEGA™ Classic, which contains a mixture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Chr. Hansen Holding A/S, Novonesis, Hoersholm, Denmark), was added (0.1% of total weight) and incubation at 40°C occurred until pH 4.5 was reached (6 hours for LPLF, 8 hours for HPHF). The gurt was then stirred, packed into plastic Minigrip bags, and stored at 4°C for 14 days. The chemical composition of the gurts was investigated at different stages of processing (after mixing, after fermentation and after storage for 14 days).

Finally, the HPHF and LPLF gurts were compared with an 'upscaled gurt' developed within the HealthFerm project and used in an intervention trial in WP3 of the project, as well as with a similar but non-fermented 'oat porridge'. The sensory characteristics of all four samples were analysed.

2.4.2 Response surface modelling

Using CCF design and response surface modelling, it was possible to investigate the effect of fermentation conditions (time, temperature, and inoculum ratio) on acidity (pH and TTA), viscosity, and β-glucan content. MODDE® 13 software (Sartorius Corporate Administration, Göttingen, Germany) was used to compute the model.

2.4.3 Multivariate analysis

PCA was used to examine clustering patterns between LPLF and HPHF across different processing stages (n = 6) and to investigate relationships among a set of 40 variables. The analysis was performed using the Singular Value Decomposition (SVD) algorithm, with samples included as dummy variables,

and full cross-validation was employed. The data were mean-centred and weighted based on standard deviation. The Unscrambler® X software (Version 10.5, Aspen Technology® Inc., USA) was used to conduct the analysis.

A PLS model was used to investigate the relationship between chemical changes and sensory characteristics of HPHF, LPLF, porridge, and gurt. For the PLS model (n=4), 15 variables (x) were: pH, TTA, viscosity, and content of fat, glucose, sucrose, fructose, maltose, total starch, total fibre, total protein, and β-glucan, and M_w of β-glucan, total protein, and aqueous extract protein. Variables (y) were 21 sensory attributes: lightness, thickness, lumpiness, overall odour intensity, flavour of fruity, cardboard, cooked starch, cereal, sour milk, and coarse texture, watery texture, flavour of cereal, sour milk, beany, fruity, and overall taste intensity, and finally sweetness, bitterness, umami, astringency, and overall aftertaste intensity. The Unscrambler® X software (Version 10.5, Aspen Technology® Inc., USA) was used to conduct the analysis.

2.5 Solid food systems – tempeh from oat and faba bean

2.5.1 Experimental setup

This study investigated how pre-treatments (soaking and boiling) with and without *Lactobacillus plantarum* and subsequent solid-state fermentation with *Rhizopus oligosporus* affect the physicochemical properties and *in vitro* digestibility of tempeh-like products made from faba bean and wholegrain oats. Samples included raw materials (R1 and R2), samples soaked with and without Lactobacillus plantarum and boiled (B1-B4) and fermented products produced via solid-state fermentation using *Rhizopus oligosporus* (T1-T4). Figure 2 shows an overview of the study where samples were analysed for nutritional composition, anti-nutritional factors, free amino acids, and an in vitro digestion protocol (INFOGEST) to measure protein and starch degradation as well as viscosity of the digesta. On the other hand, select tempeh products were prepared for sensory analysis testing by trained panellists and for analysis of volatile organic compounds and texture profile analysis. Prior to sensory evaluation, all food samples underwent microbiological analysis to assess colony-forming units (CFU), with a specific focus on Staphylococcus aureus and Bacillus cereus, to ensure the safety of the participants.

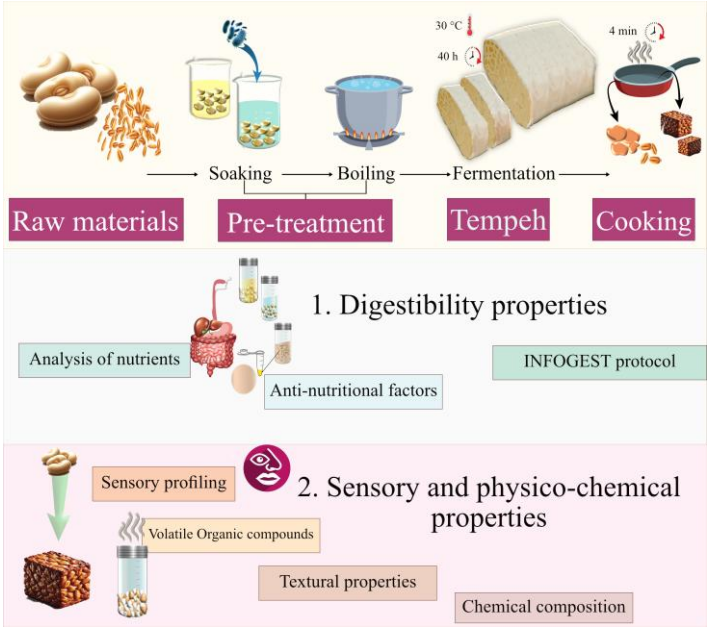

Figure 2. Overview of the experimental design used to assess the effects of fermentation on faba bean and faba–oat products, and their impact on digestibility and sensory properties.

2.5.2 Multivariate analysis

PCA was employed to investigate the impact of pre-treatment and fermentation on the nutritional composition, anti-nutritional factors, and digestive properties of oat–faba bean-based tempeh-like products. The model was constructed to explore clustering patterns among raw materials, pre-treated samples (soaked with or without *Lactobacillus plantarum*), and Tempeh fermented samples produced through solid-state fermentation (SSF) with *Rhizopus oligosporus*. Variables included in the PCA reflected comprehensive indicators of nutritional quality and digestibility, comprising total protein, fat, resistant starch, digestible starch, dietary fibre fractions (insoluble dietary fibre, IDF; soluble dietary fibre precipitated in ethanol, SDFP; and soluble dietary fibre soluble in ethanol, SDFS), ash, phytic acid, vicine, convicine, total phenolic content, and selected free amino acids (His, Ser, Arg, Gly, Asp, Glu, Thr, Ala, Pro, Lys, Tyr, Val, Ile, Leu, Phe). The in vitro digestion protocol followed the INFOGEST protocol (Brodkorb et al., 2019) and analysed samples only at the end of the gastrointestinal phase, including viscosity of the digesta, degree of protein hydrolysis (DH) with OPA method, and the release of glucose and maltose as the starch degradation. Data dimensionality was reduced to the two principal components explaining the highest variance. Model validity was assessed using R^2X (explained variance) and Q^2 (predictive ability). All analyses were conducted using SIMCA 17.0 (Umetrics, Sweden).

A PLS regression model was applied to explore the relationships between physicochemical properties and sensory attributes of faba bean and faba-oat-based tempeh-like products. The predictor variables (X), totalling 39, encompassed diverse chemical and physical characteristics. These included nutritional components such as protein, fat, resistant and non-resistant starch fractions, dietary fibre fractions, ash, and moisture content, anti-nutritional factors such as phytic acid, vicine, and convicine and some free amino acids. Volatile organic compounds were grouped into categories, including aldehydes, ketones, esters, alcohols, acids, and pyrazines. Other volatiles were quantified as well. Texture profile analysis, such as hardness, adhesiveness, cohesiveness, springiness, and chewiness, completed the set of predictor variables.

The response variables (Y) consisted of 19 sensory attributes generated using Generic Descriptive Analysis, with a semi-trained panel (N = 8, 5 females) from the Food Sciences unit at the University of Turku. Sensory profile included descriptors such as overall odour intensity, flour-like odour, fermented odour, beany odour, buttery odour, nutty odour, sweet odour, ethanol odour, musty odour, as well as taste and mouthfeel attributes including sweetness, sourness, bitterness, astringency, umami, graininess, crumbliness, chewiness, and softness. All analyses were conducted using SIMCA 17.0 (Umetrics, Sweden).

2.6 Solid food systems – extruded meat alternative

2.6.1 Experimental setup

In this study, in the context of extruded meat alternatives, faba protein concentrate (FPC) was subjected to lactic acid bacteria fermentation and subsequently dried using three different methods: oven drying (O-FF), freeze drying (F-FF), and drum drying (D-FF). Non-fermented FPC (Faba-C) was included as a control. The fermented and differently dried FPC samples were combined with pea protein isolate (PPI) in high-moisture extrusion to produce meat analogues. The impact of the drying method on functional, textural, and sensory properties, as well as on in vitro digestibility, was evaluated in both the raw materials and the meat analogues.

2.6.2 Multivariate analysis

Statistical analyses were performed to examine clustering patterns (PCA). This was done for both the differently processed FPC (Faba-C, O-FF, F-FF, D-FF) and the resulting extruded meat alternatives made

from the mix of each FPC and PPI. Different models for FPCs and extrudates were used as the measured variables were different. After confirming that the sample discrimination was adequate, the main focus was on modelling the relationships between instrumental and chemical versus sensory variables. This was studied further in a supervised model (PLS regression analysis).

Two PCA models were made to examine clustering patterns across different sample types spanning fermentation and drying types (n = 4) and to investigate relationships among a set of 33 (FPC) or 32 (extrudates) variables that spanned the functional, textural and sensory properties. The analyses were performed using the NIPALS algorithm, with samples included as downweighted dummy variables, and full cross-validation was employed. The data were mean-centred and weighted based on standard deviation. The Unscrambler® X software (Version 10.5, Aspen Technology® Inc., USA) was used to conduct the analysis.

Two PLS models were used to investigate the relationship between chemical and instrumental analyses and sensory characteristics of the FPC and extrudate samples. For the FPC model, the 20 predictor (X) variables were DSC, water binding capacity, protein solubility, protein content, dry matter content, pH, final viscosity, particle size D50 (dry and wet), L*, a* and b* colour values, free 5' nucleotides (sum of all 5' nucleotides and separately IMP, AMP, GMP contents), and free amino acids (sum of all free amino acids and separately Asp and Glu), and the equivalent umami concentration (EUC) calculated from these values using Yamaguchi's EUC formula. The response (Y) variables were the intensities of 13 sensory attributes: pea odour, roasted odour, cereal odour, sour odour, sweet odour, fruity odour, umami, roasted flavour, fermented flavour, sweetness, beany flavour, bitterness, and astringency.

For the extrudate model, the 13 predictor (X) variables were the four variables from Texture Profile Analysis (TPA; hardness and chewiness measured both when fresh and after 162 days of storage), free 5' nucleotides (sum of all 5' nucleotides and IMP, AMP, GMP contents), free amino acids (sum of all free amino acids and separately Asp and Glu), and the equivalent umami concentration (EUC). The response (Y) variables were the intensities of 20 sensory attributes: pea odour, roasted odour, cereal odour, vinegar odour, sweet odour, darkness, tearing force, elasticity, toughness, fibril length, crumbliness, umami, beany flavour, bitterness, roasted flavour, fermented flavour, spicy flavour, fracturability, residual fibrousness, and moist mouthfeel.

The PLS models were created using the Kernel PLS algorithm. Like with the PCA models, the samples were included as downweighted dummy variables, and full cross-validation was employed. The data were mean-centred and weighted based on standard deviation. The Unscrambler® X software (Version 10.5, Aspen Technology® Inc., USA) was used to conduct the analysis.

2.7 Solid food systems – wheat bread with faba bean sourdough

2.7.1 Experimental setup

The goal of this experiment was to investigate the effect of the microbial composition of faba bean flour-based sourdoughs on the sensorial, techno-functional and health-related properties of wholemeal wheat bread. In total, 8 types of bread were prepared, which differed in their raw material composition and in the microbial composition of the sourdough (Table 4). Faba bean flour was selected to increase protein content and quality of the obtained bread loaves. All breads were produced using the same process, which consisted of a bulk fermentation for 30 min at 21°C followed by a final proofing phase overnight (12 h) at 15°C before baking. Two control breads (bread 1-2) were included without the addition of sourdough of which one consisted solely of wholemeal wheat flour and the other also contained faba bean flour. Both of these breads were fermented by traditional Baker's yeast. Next, breads 3-4 were prepared with faba bean flour sourdough containing a classic *L. plantarum* and *S. cerevisiae* strain. However, the classic *S. cerevisiae* strain was not able to grow well and produce

sufficient leavening capacity in the faba bean flour sourdough. Therefore, additional baker's yeast needed to be added to obtain a good bread volume. Breads 5-8 were produced using the innovative faba bean flour sourdoughs with a unique combination of one lactic acid bacteria and one yeast strain. These strains were selected mainly based on their microbial peptidase and phytase activity and the ability to thrive well in the faba bean flour matrix. The pH, TTA and organic acid content of the sourdoughs were analysed.

The breads described in Table 4 were analysed for a range of physicochemical and nutritional properties. Measurements included water-extractable arabinoxylan content, phytate degradation, fructan content, free and total phenolic acids, free amino acids, and *in vitro* starch and protein digestibility. Bread volume, pH, total titratable acidity, and organic acid content were also determined.

Table 4. Composition of wholemeal breads incorporating faba bean flour or faba bean flour sourdough prepared with various yeast and lactic acid bacterial strains.

Bread sample	Raw material	Yeast strain	Lactic acid bacterial strain	Additional Baker's yeast added in bread recipe
Bread 1	100 % wholemeal flour	/	/	0.2%
Bread 2	100 % wholemeal flour + 11 % faba bean flour	/	/	0.2%
Bread 3	100 % wholemeal flour + 22 % faba bean flour sourdough	*S. cerevisiae*	*L. plantarum*	0.1%
Bread 4	100 % wholemeal flour + 22 % faba bean flour sourdough	*S. cerevisiae*	*L. plantarum*	/
Bread 5	100 % wholemeal flour + 22 % faba bean flour sourdough	*S. cerevisiae* KFAY2	*C. paralimentarius* CPUR-C17-01	/
Bread 6	100 % wholemeal flour + 22 % faba bean flour sourdough	*S. cerevisiae* KFAY2	*L. plantarum* TOPL1	/
Bread 7	100 % wholemeal flour + 22 % faba bean flour sourdough	*K. unispora* KFBY1	*C. paralimentarius* PUR-C17-01	/
Bread 8	100 % wholemeal flour + 22 % faba bean flour sourdough	*K. unispora* KFBY1	*L. plantarum* TOPL1	/

2.7.2 Multivariate analysis

PCA was carried out to investigate relationships between physicochemical parameters and to identify clustering patterns across bread types. The analysis was performed in JMP Pro 17 (SAS Institute, Tervuren, Belgium) using the Singular Value Decomposition (SVD) algorithm. Data were mean-centred and scaled to unit variance.

2.8 Joint model for *in vitro* protein digestibility

To investigate how different fermentation parameters (time, temperature, type of microorganisms, single strain or consortia fermentation) affected the degree of protein hydrolysis (DH%), PLS regression was performed using Unscrambler® X software (Version 10.5, Aspen Technology® Inc., USA).

The fermentation conditions were treated as predictor variables (x), while DH% after INFOGEST 2.0 protocol applied on food models developed in WP2 were used as the response variable (y). Food models included in this analysis are reported in Table 5.

Table 5. List of samples included in the analysis and respective codes.

Sample	Samples code
GURT	Gurt
Porridge	Gurt_C
CPBB-B1	Drink_1
CPBB-B2	Drink_2
CPBB-B3	Drink_3
CPBB-T1	Drink_4
CPBB-T2	Drink_5
CPBB-T3	Drink_6
CPBB-G	Drink_7
CPBB-Unstarted	Drink_8
CPBB-Raw	Drink_C
Faba-C+PPI extrudate	Extrudate_C
O-FF+PPI extrudate	Extrudate_1
F-FF+PPI extrudate	Extrudate_2
D-FF+PPI extrudate	Extrudate_3
O-FF+PPI flour	Mixture_1
F-FF+PPI flour	Mixture_2
D-FF+PPI flour	Mixture_3
Faba-C+PPI flour	Mixture_C
Tempeh 100% faba fermented	Tempeh_1
Tempeh 100% faba + LAB fermented	Tempeh_2
Tempeh 85% faba+15% oat fermented	Tempeh_3
Tempeh 85% faba+15% oat LAB fermented	Tempeh_4
Pre-treated 100% faba	Tempeh_C1
Pre-treated 100% faba + LAB	Tempeh_C2
Pre-treated 85% faba+15% oat	Tempeh_C3
Pre-treated 85% faba+15% oat LAB	Tempeh_C4

3. Results

3.1 Raw materials – faba bean ingredients

PLS regression modelling was applied to examine the relationship between potential flavour-active metabolites and sensory properties (Figure 3).

In this model, 96% of the variation in metabolites explained 88% of the variation in the sensory data across the two factors. Only a few compounds closely linked to sweetness, including protocatechuic acid, histidyllysine, glucotropaeolin, and two unknown compounds, and these were mainly associated with faba bean flour (FF). Several metabolites were associated with bitterness, mouth-drying orosensation, and strong taste and aftertaste intensities, predominantly in the faba bean protein concentrates (FPC). The largest group of these were carbohydrates and carbohydrate conjugates such as 2,3-butanediol apiosylglucoside, verbascose, vicine, and convicine. The second largest group included lipids of various types, including terpene and fatty acyl glycosides, prenol lipids, and fatty acids. Additionally, metabolites such as lysine, histidylserine, oxidised glutathione, γ-L-glutamyl-S-(1-propenyl)-cysteine sulfoxide, derivatives of aspartic acid, jasmonyl-leucine or isoleucine, organoheterocyclic caprolactam, and L-4-chlorotryptophan were also linked to bitterness. Compounds

such as tripeptides, oligonucleotides, and nucleotides were associated with umami taste, which was primarily observed in the faba bean protein isolate (FPI).

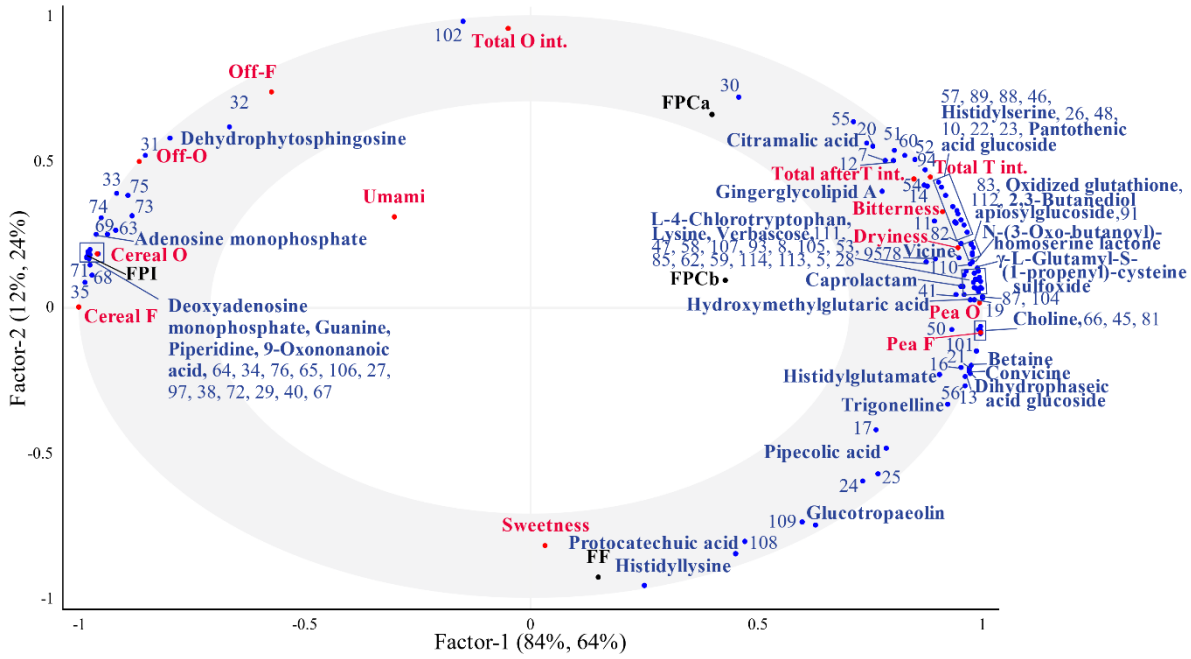

Figure 3. Partial least squares regression (PLS) plot illustrating the relationship between annotated metabolites (N = 115) (predictors, shown in blue) and sensory attributes (N = 13) (responses, shown in red) for faba bean flour (FF), faba bean protein concentrates (FPCa, FPCb), and faba bean protein isolate (FPI) (shown in black).

3.2 Liquid food systems – cereal-pulse-based beverages

A PCR biplot was constructed to investigate the relationships between the individual coordinates of CPBB samples and sensory descriptors based on projective mapping data (Figure 4A). A total of 37 sensory descriptors were used, twelve of which were spontaneously provided by the panellists. Most frequently used sensory attributes to describe CPBB samples were “Cereal” (the sensory attribute has been selected 369 times), “Dairy” (244), “Sour” (243), and “Sweet” (232), which are somewhat consistent with sensory attributes for fermented dairy products. The first principal component (PC-1) explained 33% of the variation in sample individual coordinates and accounted for 60% of the variability in sensory descriptors, while PC-2 captured 19% of sample variation, corresponding to 16% of sensory descriptor variability. This PCR model successfully enabled to differentiate four distinct clusters of CPBB samples, with CPBB-Raw and CPBB-Unstarted forming individual grouping, each showing diverse sensory characteristics. Key sensory descriptors contributing to sample differentiation included “Sweet” and “Dairy” for CPBB-Raw and “Grassy” and “Oat” for CPBB-Unstarted. Specific fermented samples showed strong associations with descriptors such as “Fruity,” “Cardboard,” and “Pear” (e.g., CPBB-B1, CPBB-B2), while others were linked to “Beany,” “Potato,” and “Raw bean” (e.g., CPBB-B3, CPBB-T2).

Liking scores followed a consistent pattern, with CPBB-Raw and fermented samples receiving the highest ratings, approaching the threshold for positive consumer acceptance. In contrast, CPBB-Unstarted samples consistently scored the lowest, with taste and overall liking falling just below neutral.

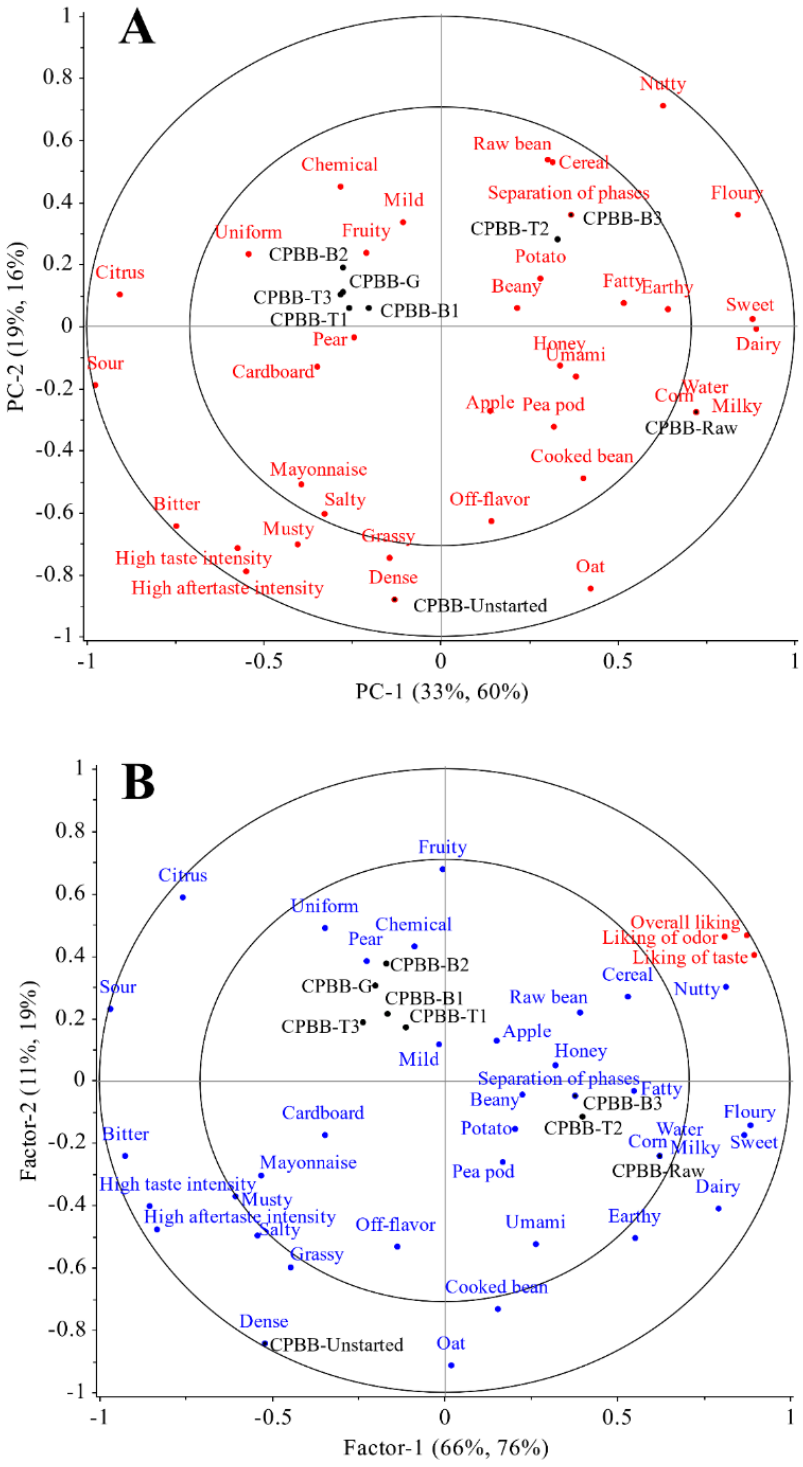

Figure 4. Sensory evaluation of raw cereal-pulse-based beverage (CPBB-Raw), cereal-pulse-based beverage without bacterial inoculum (CPBB-Unstarted), and CPBB-Started, which were incubated for 20 h at 30°C. Fermentation (CPBB-Started) was performed using defined microbial consortia structured into binary (CPBB-B1, B2, B3), ternary (CPBB-T1, T2, T3), and quaternary (CPBB-G) cultures. Principal component regression (PCR) biplot for individual coordinates of beverages (X, not shown in the figure) and sensory descriptors (Y) of projective mapping. Beverages are shown as black dots and descriptors are represented by red dots. Principal components (PC) 1 and 2 are shown as x- and y-axes, respectively (Panel A). Partial least squares (PLS) regression biplot applied to sensory descriptors (predictors, X), and liking ratings (responses, Y) of projective mapping. CPBB samples are shown as black dots, descriptors as blue dots, and liking (of odour, taste, and overall) is represented by red dots. Factors 1 and 2 are shown as x- and y-axes, respectively (Panel B).

To better understand and predict consumer liking based on sensory attributes, a PLS regression biplot was applied (Figure 4B). The model captured 66% of the variation in sensory descriptors and explained 76% of the variation in liking ratings on Factor-1, while Factor-2 accounted for 11% of descriptor variation and 19% of liking ratings variation. Sensory descriptors such as “Cereal,” “Nutty,” “Apple,” and “Raw beans” were strong positive predictors of liking (odour, taste, overall), while “High taste intensity,” “High aftertaste intensity,” and “Bitter” were negatively associated with consumer preferences. Sample clustering in the PLS model closely aligned with PCR results, except for CPBB-B3 and CPBB-T2, which grouped with CPBB-Raw.

The application of multivariate analyses like PCR and PLS helped clarify how different microbial consortia and their interactions influenced the sensory properties and clustering of the CPBB samples. The alignment between the two models confirmed their impact in shaping both product characteristics and consumer liking.

3.3 Liquid food systems – oat-based drink model system

Figure 5 presents a colour map of the correlations among all measured parameters for the oat-based drink model system. Four correlations were found to be statistically significant ($p < 0.05$). Notably, higher protein extractability was associated with increased β-glucan extractability and vice versa. Additionally, higher average protein molecular weights correlated with increased viscosity and decreased ζ-potential. The creaming index showed a strong negative correlation with extract viscosity, meaning that the higher the viscosity, the more stable the O/W emulsion will be. No other parameters were significantly correlated with this creaming index. Similarly, no significant correlations were observed between any measured parameters and the average oil droplet size. Interestingly, some expected relationships—such as between protein extractability and average protein molecular weight, or between viscosity and β-glucan extractability—were not detected. This suggests that the current dataset may be insufficient to capture these trends, and that additional data points or incubation conditions are likely needed to improve the robustness of the correlation analysis in this liquid model system.

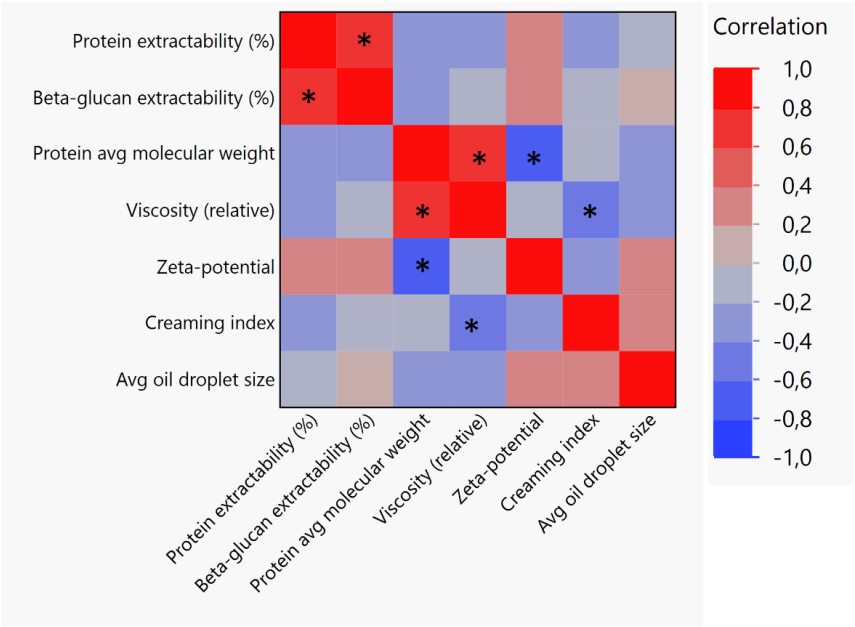

Figure 5. Colour map on correlations for the emulsion model. Statistically significant correlations ($p < 0.05$) are indicated with an asterisk.

3.4 Semi-solid food systems – gurt from oat and faba bean

3.4.1 Response surface model of pH, TTA and viscosity

For the gurt made with oat and faba bean, the models for pH, TTA, and viscosity showed good significance and predictability. For pH, both R^2 and Q^2 values were above 0.5, with a difference below 0.3, indicating the model was reliable for future predictions. Reproducibility was high, showing low variation among replicates, and although model validity was below 0.5, it was still above 0.25, confirming that the model error was not larger than the pure error. The TTA model performed similarly, with R^2 and Q^2 significantly above 0.5, a difference below 0.3, high reproducibility (0.87), and a higher model validity (>0.75) compared to pH, confirming its robustness.

The viscosity model was the strongest, with R^2 and Q^2 values close to 1, indicating high significance and excellent predictability. The difference between these parameters was less than 0.3, reproducibility was high, and model validity was slightly above 0.5, confirming that the data accurately represented the model. In contrast, β-glucan content could not be modelled because no significant coefficients were obtained, largely due to the lack of variation between samples. After removing insignificant coefficients, no terms remained significant, and thus no regression equation for β-glucan was derived.

The regression equations for pH, TTA, and viscosity are reported as follows:

$$pH = -2.0 - 9.4 \times 10^{-5} * t - 5.9 \times 10^{-5} * T - 3.9 \times 10^{-3} * I + 6.2 \times 10^{-4} * I^2 + 9.3 \times 10^{-5} * (tI) + 8.0 \times 10^{-5} * (TI)$$

$$TTA = 0.5 + 0.2 * t + 0.1 * T + 1.1 * I$$

$$Viscosity = 6.5 + 0.2 * t - 0.4 * T + 3.9 \times 10^{-2} * I - 1.3 \times 10^{-3} * t^2 + 6.2 \times 10^{-3} * T^2 + 0.2 * I^2 + 4.2 \times 10^{-3} * (tT) + 3.1 \times 10^{-2} * (tI)$$

$Where$:
$t = fermentation\ time\ (hours, h)$
$T = fermentation\ temperature\ (°C)$
$I = inoculum\ ratio\ \left(\%\frac{w}{w}\right)$
$t^2, T^2, I^2 = quadratic\ (squared) effects\ of\ time, temperature, and\ inoculum\ ratio$
$t \times I, T \times I, t \times T = interaction\ terms\ between\ the\ factors$

All fermented samples showed a marked decrease in pH compared to the non-fermented (NF) control (6.25 ± 0.03), with pH values ranging from 5.64 to 4.18, most remaining below 5. Increasing fermentation time, temperature, and inoculum ratio each significantly reduced pH, with the squared effect of inoculum and its interactions with time and temperature further amplifying this decrease, indicating a non-linear relationship (Figure 6). Correspondingly, TTA increased with fermentation, ranging from 3.62 ml to 10.07 ml compared to 2.01 ± 0.07 ml in NF samples. For TTA, only the individual linear effects of time, temperature, and inoculum were significant, each contributing to increased acidity, while squared and interaction terms were not significant.

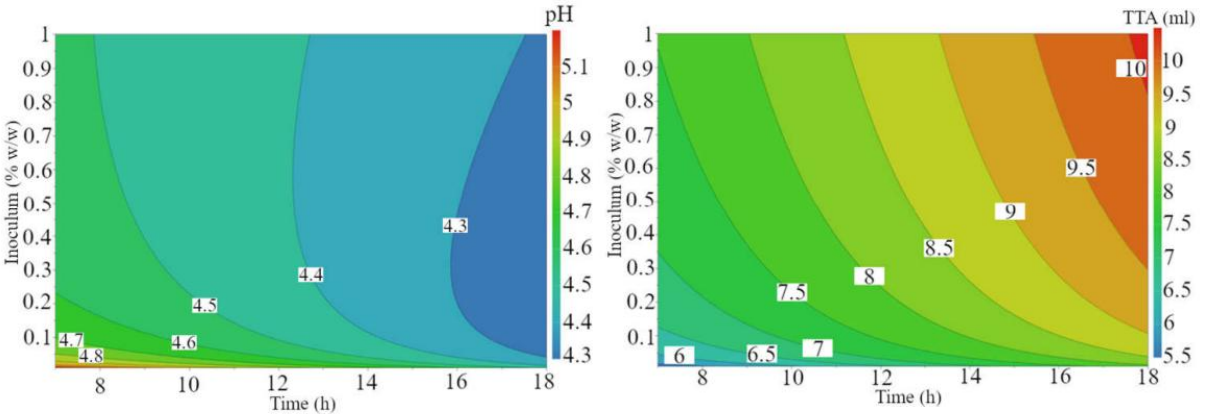

Figure 6. Response contour plots on the effects of time and inoculum on pH (on the left) and TTA (on the right) of fermented oat and faba bean mixture at 37.5 °C. Numbers on the plot indicate the pH and TTA values predicted at specific fermentation time and inoculum ratio.

Viscosity of fermented samples (Figure 7) ranged from 1.05 to 3.89 Pa·s compared to 2.59 ± 0.15 Pa·s in the NF sample. Temperature had the greatest impact on viscosity (coefficient 0.98), followed by time (−0.17) and inoculum ratio (0.09), and all factors showed non-linear effects, as evidenced by significant squared terms. The viscosity model contained the highest number of quadratic and interaction terms compared to pH and TTA, although the combined effect of temperature and inoculum ratio was not significant. In contrast, β-glucan content could not be modelled as no significant coefficients were obtained; only the squared effect of time appeared significant, but it was insufficient to establish a reliable model.

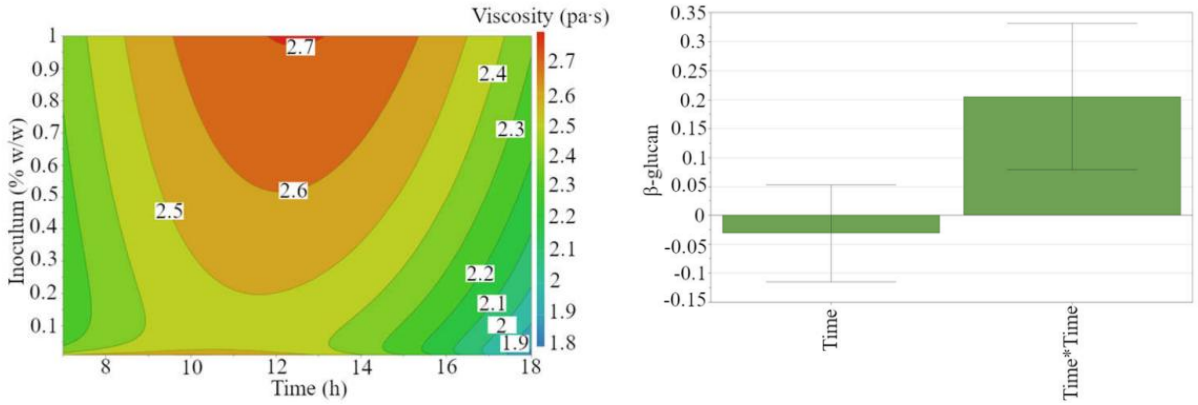

Figure 7. On the left, response contour plot on the effects of time and inoculum on viscosity of fermented oat and faba bean mixture at 37.5 °C. Numbers on the plot indicate the viscosity predicted at specific fermentation time and inoculum ratio. On the right, coefficient plot for β-glucan content.

In conclusion, across all tested fermentation conditions (time, temperature, inoculum ratio), no significant reduction in β-glucan content was observed. However, relationships between the fermentation factors and other responses were identified. All factors led to a decrease in pH and an increase in TTA. While all factors influenced viscosity, temperature was significantly more impactful than the others, showing a positive, non-linear effect.

3.4.2 Multivariate analysis of physicochemical properties of HPHF and LPLF gurts

The key findings from the characterisation of LPLF and HPHF samples were analysed and summarised using PCA. The bi-plot in Figure 8 shows the placement of LPLF and HPHF samples after mixing, fermentation, and 14 days of storage. PC1 and PC2 explained 89% of the total variance, showcasing distinct differences between LPLF and HPHF along PC1. LPLF was associated with higher levels of fructose, sucrose, fat, histidine, β-glucan Mw, and lightness (L*). HPHF was associated with higher

concentrations of maltose, glucose, starch, fibres, proteins, all other FAAs, increased acidity, viscosity, and greater yellowness (b*) and redness (a*). Additionally, the samples exhibited differentiation along PC2, clearly separating the initial mixing phase from the samples obtained after fermentation and 14 days of storage. The PCA results confirmed that changes in formulation and processing affected the physicochemical properties, resulting in variations between LPLF and HPHF, as well as at different processing stages.

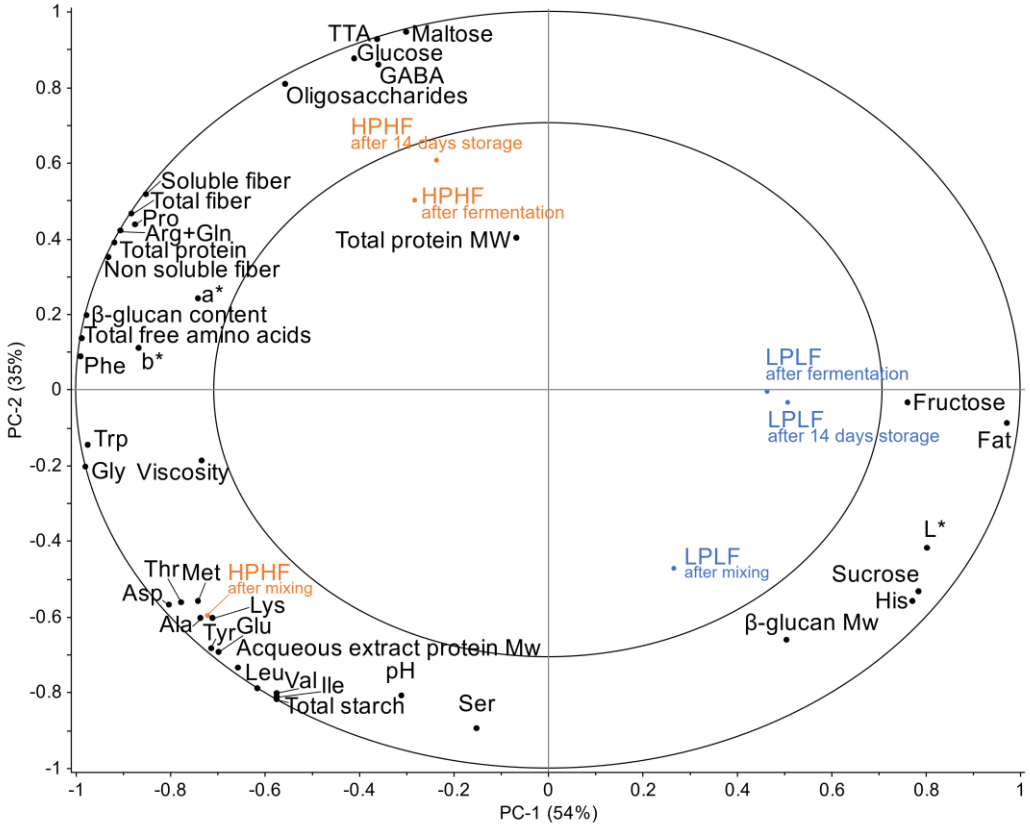

Figure 8. Principal component analysis (PCA) of the average measurements for low-protein, low-fibre control (LPLF, blue) and high-protein, high-fibre gurt (HPHF, orange) at different stages of processing.

3.4.3 Multivariate modelling of sensory profiles in gurts using PLS

A PLS model was built to investigate the effect of fermentation-induced changes on sensory attributes in LPLF, HPHF, upscaled gurt, and porridge (Figure 9). The PLS biplot shows that the sensory, chemical, and physical properties of samples are strongly influenced by their composition, with HPHF and LPLF samples separated along Factor 1, which explains most of the variance (45%), indicating distinct associations between chemical composition (e.g., β-glucan, protein, sugars) and sensory attributes (e.g., taste, texture, aroma). The model enabled the display of three different patterns. The pH, viscosity, and total starch were strongly associated with sensory attributes, such as cereal odour and flavour, thickness, cooked starch, and lumpiness, all of which were linked with the porridge sample. Whereas maltose, glucose, and TTA were strongly associated with sweetness, overall odour and taste intensity, and overall aftertaste intensity, which were linked with HPHF and gurt samples, both of which were fermented and high in protein and fibre content. Although sucrose and fructose content were not directly associated with sensory attributes, they were linked to the LPLF mixture. This model demonstrates how fermentation and compositional differences shape the sensory profile of each sample type.

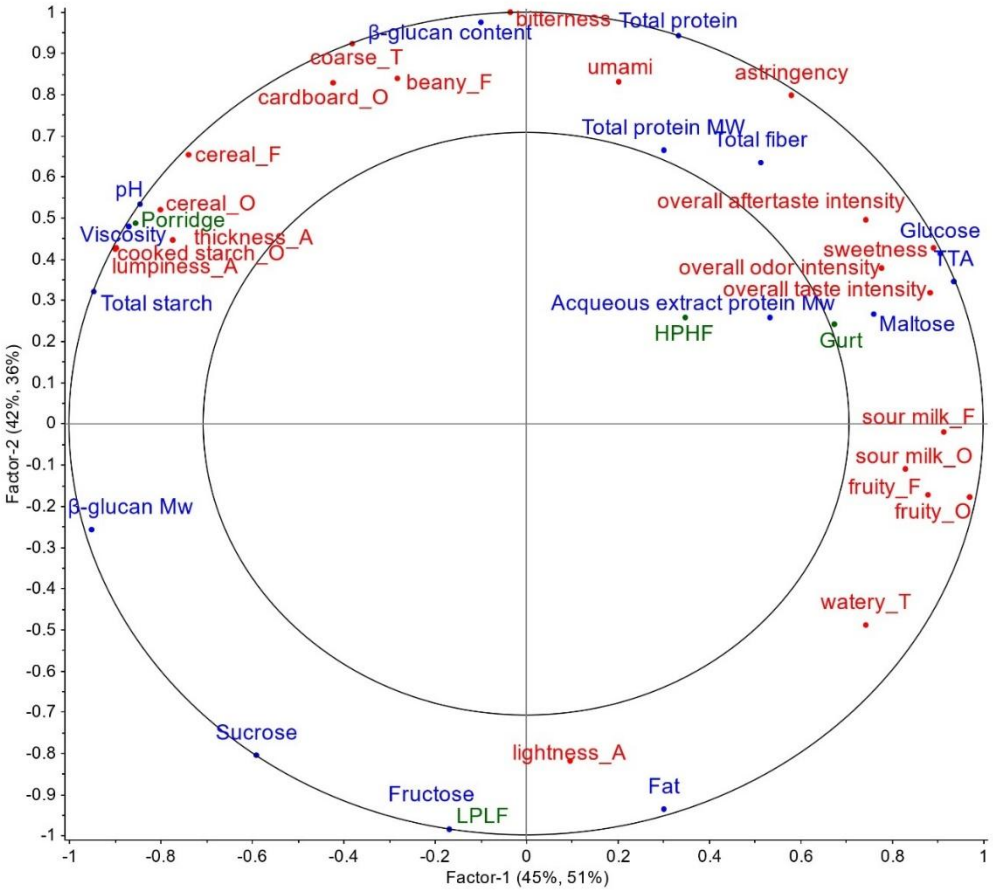

Figure 9. Partial least squares regression (PLS) loading plot for chemical changes induced by fermentation (predictors, X) and sensory attributes (responses, Y) in the food models studied (LPLF, HPHF, porridge, and gurt). Predictors are shown as blue dots, and responses are indicated by a red dot. The two percentages in parentheses next to the axis label indicate the total variance explained by the component for predictors and responses, respectively.

3.5 Solid food systems – tempeh from oat and faba bean

3.5.1 Multivariate analysis of digestibility properties of tempeh (in vitro model)

A PCA model was constructed using all nutritional, anti-nutrients and digestibility properties after in vitro digestion as X variables to explore and visualise sample groupings and identify key variables differentiating raw materials, pre-treated (soaking + boiling) and tempeh fermented products (Figure 10). The first two principal components explained 58.8% and 20.9% of the total variance, respectively. The cumulative variance captured by the model was $R^2X(cum) = 0.453$. The distance to the model (DModX) and critical value (DCrit = 1.728) values were used to detect outliers based on the distance of observations to the model plane. Figure 10 shows the A. score plots, and B. loadings provided insight into sensory-driven sample clustering.

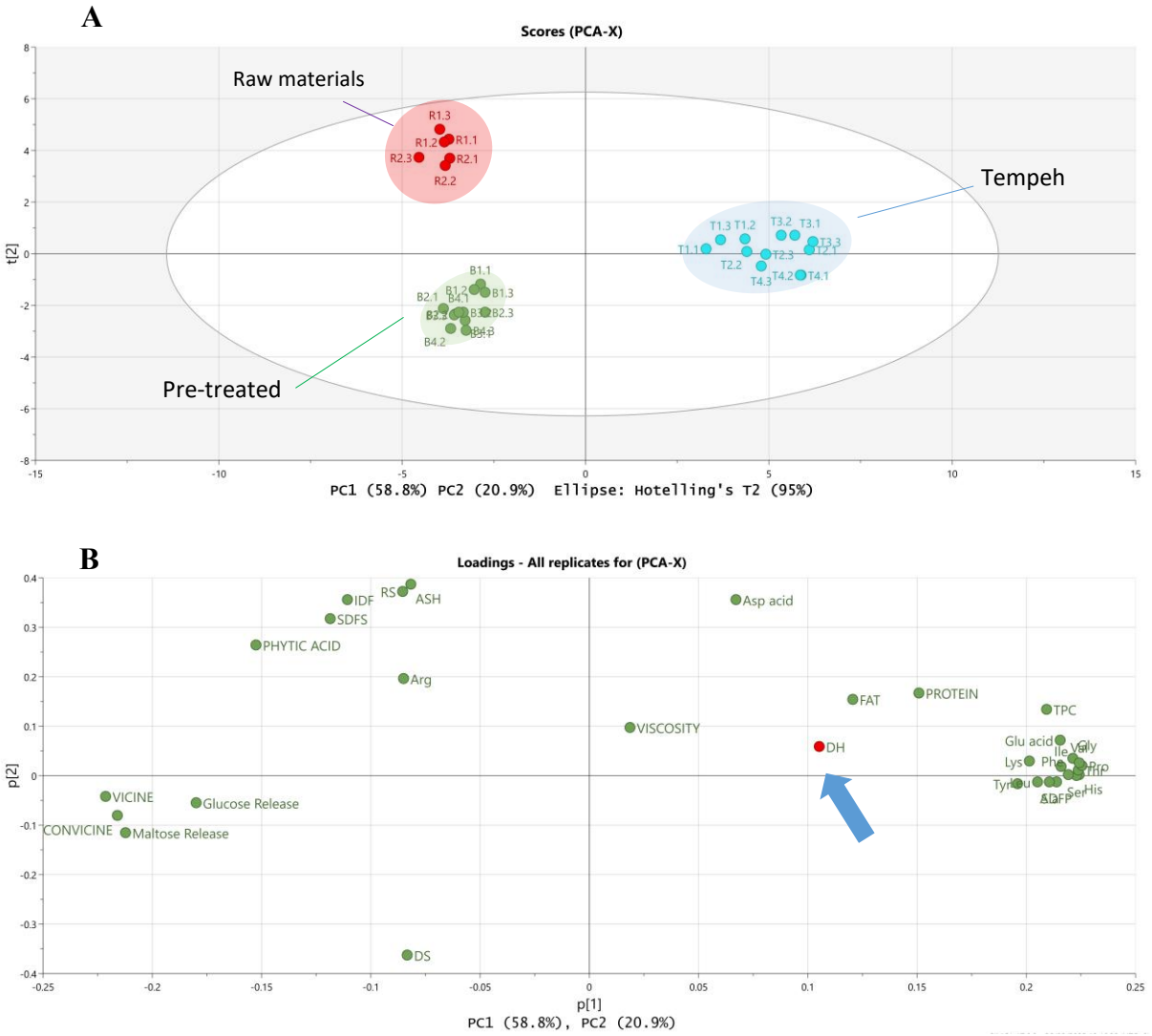

Figure 10. PCA Model based on nutritional, anti-nutritional and digestive outcomes A. Scores plots raw materials, pre-treated and tempeh samples. B. Loadings plots of the analysis on nutrients, anti-nutrients, free amino acids, and post in vitro digestion DH: degree of hydrolysis and glucose and maltose release.

The PCA scores and loadings together provide a strong multivariate insight into how processing, particularly fermentation, modifies the nutritional and anti-nutritional composition of faba bean-based ingredients. PC1 (58.8%) shows how raw samples (R1, R2) and pre-treated samples (B1-B4) load strongly on the negative side of PC1, while fermented samples (T1–T4) shift progressively toward the positive axis, indicating major compositional transformations. PC2 (20.9%) provides secondary separation, influenced by variables such as fibre fractions and specific anti-nutrients. The loading plot reveals that the degree of hydrolysis positively correlates with protein content, free amino acids (e.g., Glu, Lys, Thr), and fat, highlighting enhanced proteolysis and nutrient accessibility through fermentation. However, total phenolic content was also highly correlated despite the fact that it could potentially impair protein digestibility. In contrast, anti-nutritional factors such as phytic acid, vicine, and convicine are positioned negatively along PC1 and PC2, clustering with raw and pre-treated samples, indicating limited degradation in pre-treated material. Pre-treated samples were also correlated with elevated glucose and maltose release after in vitro digestion and proximity.

3.5.2 PLS regression model of sensory and physicochemical properties of tempeh

A PLS regression model linked sensory attributes (N = 19, Y) obtained from trained panellists to various physicochemical properties (N = 39, X), including nutritional composition (green), anti-nutritional compounds (rose), free amino acids (purple), volatile organic compounds (yellow), and texture profile parameters (blue) (Figure 11). The first two components explained 31.6% (PLS1) and 26.1% (PLS2) of the total variance, respectively.

Sensory attributes such as umami flavour and buttery, nutty, and beany odours were closely associated with free amino acids, particularly arginine, alanine, phenylalanine, leucine, and isoleucine. Volatile compounds such as alcohols were associated with ethanol odour, while esters correlated with fermented and sweet odours. Texture attributes like chewiness were strongly associated with springiness, as measured by the Texture Profile Analysis (TPA).

Free amino acids and digestible starch (DS) were positively correlated with the tempeh sample composed of 85% faba bean and 15% oat (T4), which was formulated using lactic acid bacteria (LAB) during soaking. This suggests that both raw material composition and LAB pre-treatment significantly influenced the flavour profile.

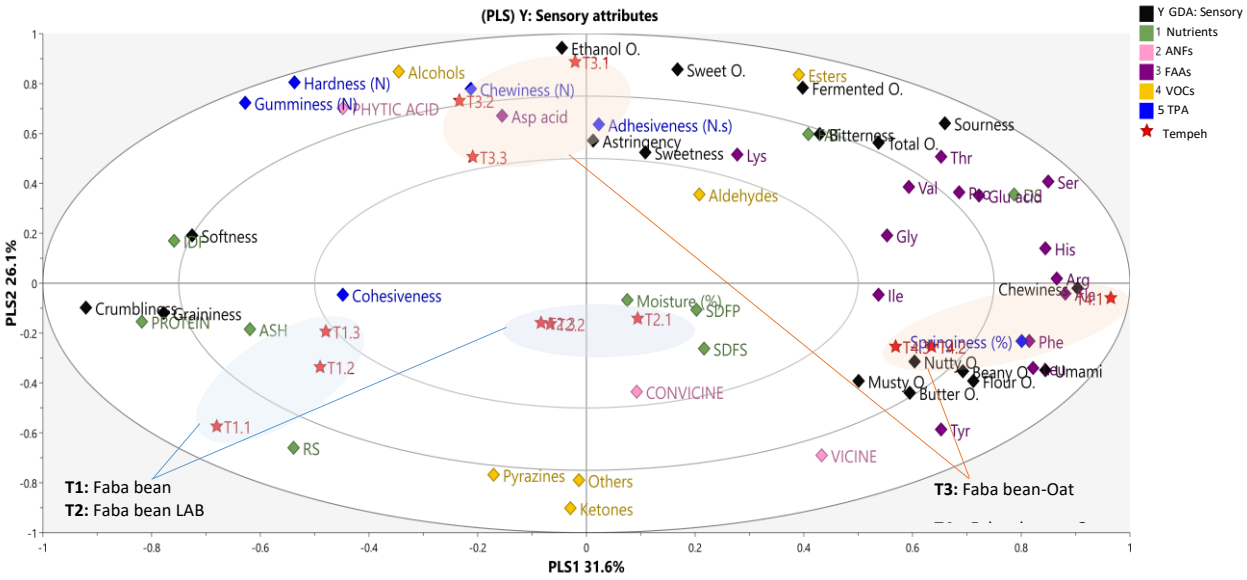

Figure 11. PLS regression plot illustrating the relationship between Y-variable: sensory scores from trained panellists and explanatory variables (X): Nutritional composition (green), Anti-nutritional factors (rose), Free amino acid profile (purple), Volatile organic compounds (yellow), and Texture profile analysis (blue).

Conversely, the 100% faba bean tempeh (T1), produced without LAB treatment, was positioned on the opposite side of T4. It was characterised by higher levels of resistant starch, insoluble and soluble dietary fibre, and protein content. Sensory attributes such as firmness, crumbliness, and graininess were prominent, indicating a denser matrix with reduced proteolytic breakdown.

Volatile compounds such as pyrazines and ketones were slightly associated with nutty and roasted odours, while aldehydes and amino acids such as lysine and aspartic acid were positively associated with sweetness and astringency. In contrast, fat content and attributes like bitterness, sourness, and total odour intensity clustered near amino acids, including threonine, valine, proline, and glutamic acid. Vicine was positioned near musty odour and close to the central cluster of anti-nutritional factors, suggesting its presence in lower concentrations compared to non-fermented faba bean products.

The PLS model highlights how targeted fermentation and raw material blending can steer flavour and texture formation, offering strategies to design plant-based foods with improved sensory appeal and functional quality.

3.6 Solid food systems – extruded meat alternative

In the context of the extruded meat alternative, PCA correlation loading plots with the samples included as dummy variables were generated to visualise sample groupings and identify key variables differentiating raw materials. As the model configuration (coordinates of the samples and variables as well as their correlations) was almost identical in the PCA model and the subsequent PLS regression model, only the latter model is shown in this deliverable.

In the FPC PLS model (Figure 12), the largest variation (PC-1, 72% explained variance for chemical and instrumental variables, 53% for sensory variables) was between non-fermented FPC (Faba-C) and all fermented FPCs (F-FF, O-FF, D-FF). Therefore, fermentation alone had a major effect on the sample properties. The control FPC was especially different from fermented and dried samples in terms of higher pH, higher final viscosity, lower dry matter content, lower values of umami-related free amino acids and the equivalent umami concentration (EUC), milder umami flavour, was of lighter colour (L* value), and was more bitter, astringent, and beany.

In PC-2 (18% and 36%), the model separated the different drying processes (freeze-dried, oven drying, drum drying) from each other. Fermentation coupled with only gentle drying (F-FF) had a more fruity and sour odour and a more fermented flavour. On the other hand, drying processes with more heat, especially in the drum-dried (D-FF) sample, resulted in the loss of these sensory attributes in favour of roasted and cereal-like flavour. The drum-dried sample was also characterised by higher water-binding capacity.

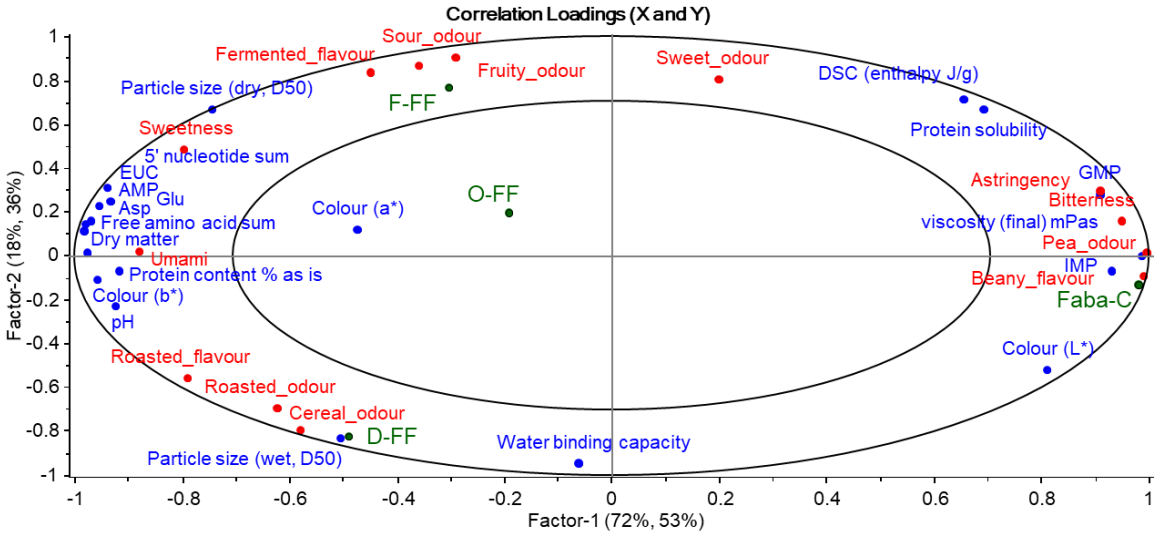

Figure 12. PLS correlation loadings plot of the FPC used for the high-moisture extrusion. The sensory attributes are marked in red in the figure.

In the corresponding extrudate PLS model (Figure 13), the overall arrangement of the samples resembled that of the FPC model: the non-fermented Faba-C sample stood out as an outlier and primarily influenced the separation along PC-1 (61% explained variance for chemical and instrumental variables, 41% for sensory variables). The non-fermented sample was again characterised by beany

odour and flavour, but with the extrudates was not considered the most beany sample. Additionally, higher perceived fracturability characterised the control extrudate.

PC-2 (38% and 25%) distinguished between the different drying processes. Notably, in the extrudates, the effect of FPC heat treatment was more pronounced, resulting in a clearer separation between oven-dried and drum-dried samples. The drum-dried extrudate closely resembled the freeze-dried extrudate, possibly because the high temperatures used in high-moisture extrusion impart a heat treatment similar to that of the brief drum-drying process applied to the FPC. Texture-related variables (both TPA and sensory) were driving separation in PC-2, with the O-FF sample having more intense perceived crumbliness and moist mouthfeel as well as larger chewiness in the TPA, but lower perceived fibril lengths, tearing force, and toughness than the D-FF and F-FF samples.

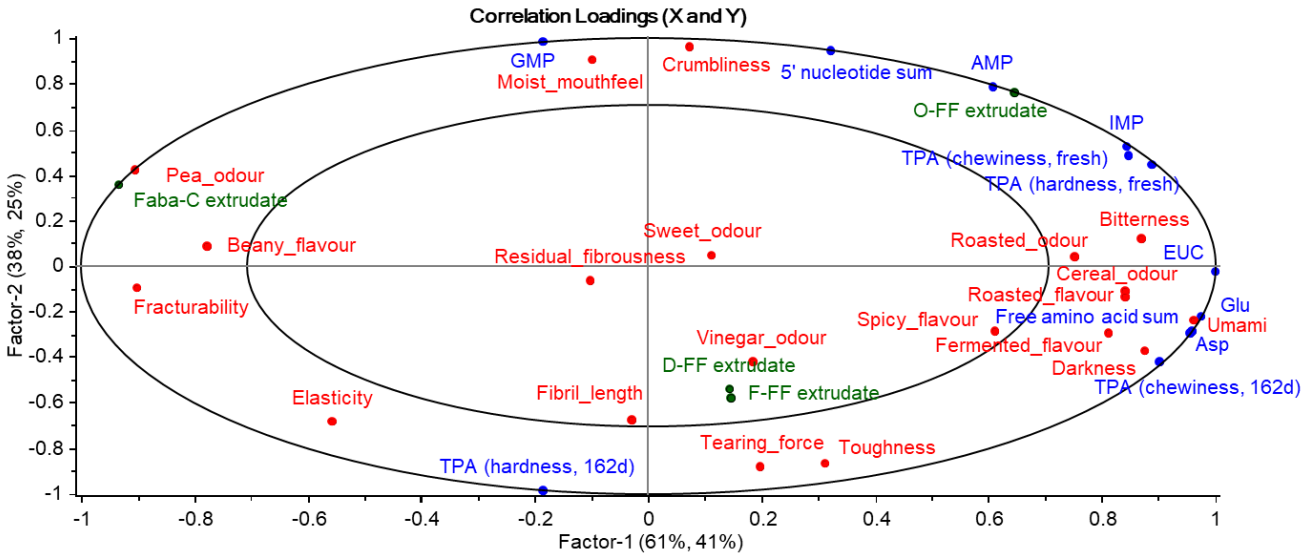

Figure 13. PLS correlation loadings plot of the extruded meat alternatives. The sensory attributes are marked in red in the figure.

When comparing the two PLS regression models, we can conclude that the umami intensity could be modelled with the equivalent umami concentration (EUC) formula, and even glutamate content alone was a working proxy indicator for umami. Colour values, especially L* and b*, and final viscosity, predicted the combined effect of fermentation heat-based drying in the FPC, while instrumental hardness measured close to the sensory profiling date predicted tactile texture properties. Overall, the PLS models showed clear differences between samples and sensory as well as chemical differences linked to the samples.

One significant limitation of these PCA and PLS models is that they are based on only four samples. As a result, although the first two principal components account for most of the variance in the dataset, the overall validity of the models is limited. There are not enough segments for robust cross-validation, leading to low Q^2 values (below 0.3). This limits the predictive aspects of the models. Nevertheless, the strong concordance observed between the FPC and extrudate models suggests a reliable and consistent interpretation of the phenomena under study, reinforcing the credibility of the sensory and analytical findings despite the limited sample size.

3.7 Solid food systems – wheat bread with faba bean sourdough

PCA was conducted to explore the variation among the eight bread samples made with faba bean sourdough based on a comprehensive set of physicochemical and nutritional parameters, including

specific volume, pH, TTA, lactic acid, total phenolics, free amino acids, phytate degradation, water-extractable arabinoxylan, fructan content, and *in vitro* starch and protein digestibility (Figure 14).

The PCA score plot (left panel) shows that the first two principal components (PC1 and PC2) explain 78.2% of the total variance (PC1: 59.7%, PC2: 18.5%). Control breads without sourdough (Bread 1 and 2, black dots) cluster together, while sourdough breads are positioned more toward the right side of the plot. No clear separation is observed between breads fermented with traditional sourdough strains (orange dots) and those with innovative strains (green dots), although Bread 8 appears to deviate from the general pattern.

The loading plot (right panel) reveals that PC1 is primarily influenced by pH, *in vitro* starch digestibility, free amino acids, TTA, lactic acid, and total phenolics. As expected, pH is negatively associated with TTA and lactic acid. The distribution of breads along PC1 thus reflects the presence and activity of sourdough fermentation. Notably, *in vitro* starch digestibility is positively associated with pH and negatively associated with TTA and lactic acid. Due to the limited number of samples (n = 8), these associations should be interpreted with caution. PC2 is mainly driven by *in vitro* protein digestibility, water-extractable arabinoxylan, and fructan content. Bread 8 is characterised by higher fructan levels but lower protein digestibility and arabinoxylan extractability, distinguishing it from the other samples. Further investigation is warranted to understand these differences.

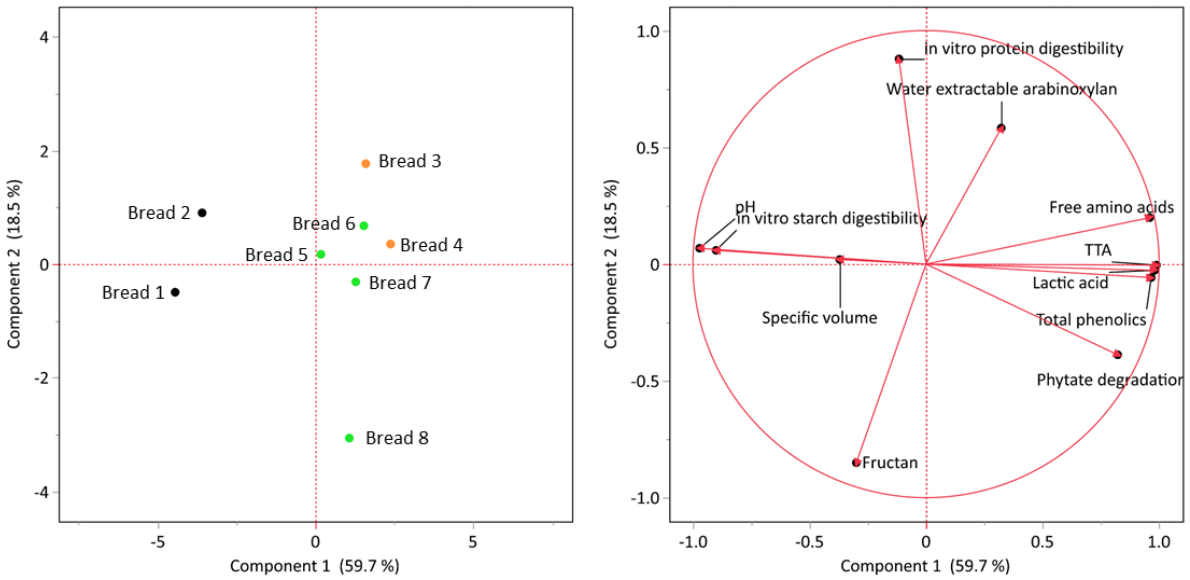

Figure 14. Principal Component Analysis (PCA) of eight bread samples based on physicochemical and nutritional parameters. Bread 1 and 2 (black dots) represent control breads without sourdough; Bread 3 and 4 (orange dots) are sourdough breads fermented with traditional strains; and Bread 5 to 8 (green dots) are sourdough breads fermented with innovative strains.

To further analyse the differences between the sourdough breads and to include measurements on the sourdoughs, another principal component analysis was performed only on the 6 sourdough breads, including the following extra parameters: pH of sourdough, TTA of sourdough, lactic acid content of sourdough and acetic acid content of sourdough (Figure 15). The PCA score plot (left panel) shows that the first two principal components (PC1 and PC2) explain 73.4% of the total variance (PC1: 45.8%, PC2: 27.6%). The sourdough breads are spread across the score plot, and no clustering is observed.

The loading plot (right panel) shows that *in vitro* starch digestibility is mostly associated with sourdough parameters (pH, TTA, acetic acid) and less with bread parameters (pH, TTA, lactic acid). This shows potential for selecting sourdoughs optimised for lower starch digestibility, although more research is needed to verify this. *In vitro* protein digestibility does not seem to be affected by sourdough parameters but is positively associated with free amino acid content.

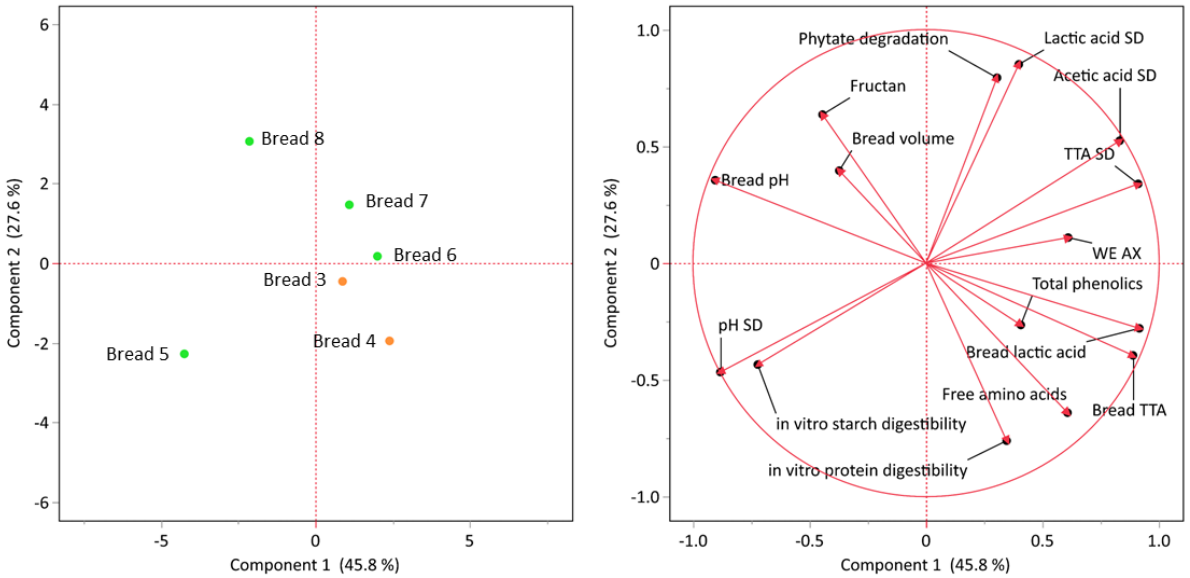

Figure 15. Principal Component Analysis (PCA) of six sourdough bread samples based on physicochemical and nutritional parameters. Bread 3 and 4 (orange dots) are sourdough breads fermented with traditional strains; and Bread 5 to 8 (green dots) are sourdough breads fermented with innovative strains. SD = sourdough; WE AX = water extractable arabinoxylan.

3.8 A joint model for *in vitro* protein digestibility

The PLS regression model made by using fermentation conditions as predictors and degree of protein hydrolysis as response from most systems described in this deliverable showed a clear, distinct pattern of fermented and unfermented samples (Figure 16). All not-inoculated and unfermented controls are located in a square and linked to the no inoculum condition, whereas fermented samples are in other squares. Additionally, tempeh samples were linked to fungi as expected. A different pattern for single strains compared to associations of two or more strains has been detected.

The DH% was strongly associated with the presence of 2 or more strains during fermentation, indicating the potential of using consortia for inducing protein hydrolysis across different food models and matrices.

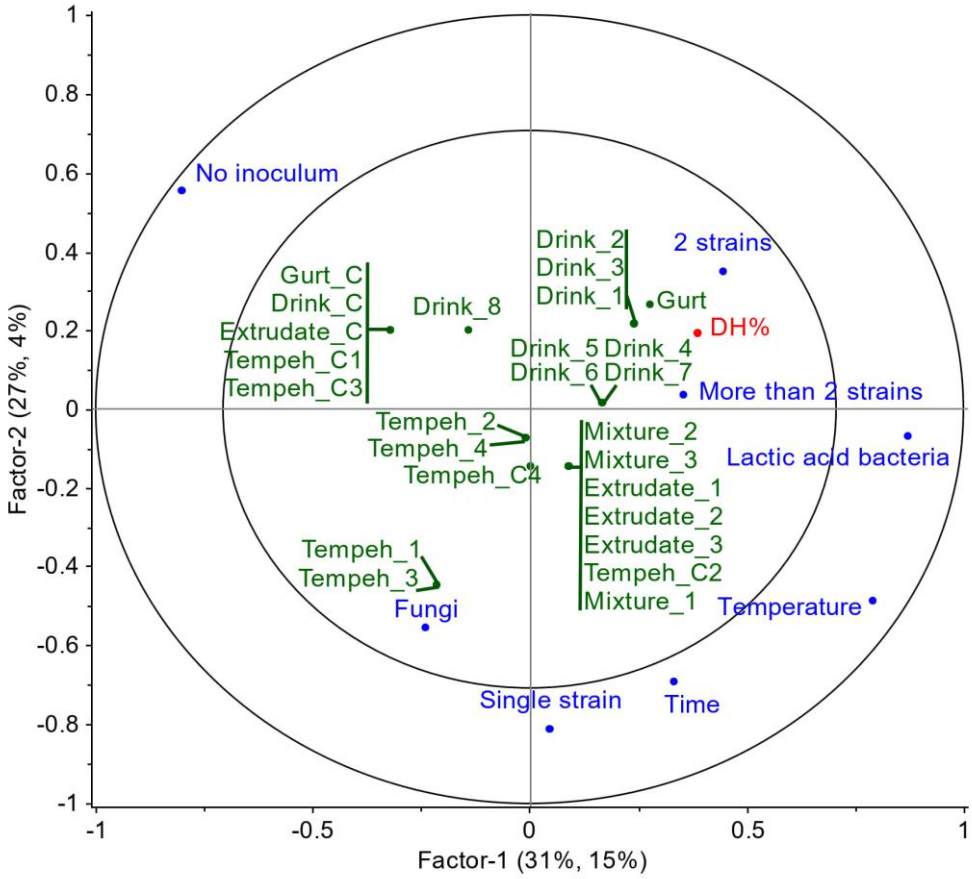

Figure 16. Partial least squares (PLS) regression loading plot for fermentation conditions (predictors, X) and degree of protein hydrolysis (DH%) after INFOGEST (responses, Y) in fermented food models. Predictors are shown as blue dots and responses by a red dot. The two percentages in parentheses next to the axis label indicate the total variance explained by the component for predictors and responses, respectively.

4. Conclusion

This deliverable applied multivariate analyses and predictive models to link fermentation conditions with health-related, sensorial, and techno-functional properties across liquid, semi-solid, and solid food systems.

In liquid systems, the oat-based drink model showed significant correlations between protein extractability, β-glucan extractability, protein molecular weight, viscosity, ζ-potential, and emulsion stability. In cereal–pulse-based beverages, PCA and PLS analyses revealed distinct clustering between fermented and non-fermented samples, with attributes such as “cereal,” “nutty,” and “apple” positively associated with consumer liking, while “bitter,” “high taste intensity,” and “high aftertaste intensity” were negatively associated.

In semi-solid systems, response surface modelling demonstrated that fermentation time and temperature were the most important factors influencing pH, titratable acidity, and viscosity in an oat–faba mixture. PCA further confirmed clear differences between LPLF and HPHF gurts across processing stages. PLS analysis highlighted that fermentation and compositional differences shaped sensory profiles in HPHF, LPLF and upscaled gurts and porridge.

In solid systems, several distinct patterns were observed. In tempeh from oat and faba bean, PCA showed a nutritional shift from raw and pre-treated samples to fermented products, with enhanced proteolysis, greater release of free amino acids, and improved nutrient accessibility. Anti-nutritional factors clustered with raw and soaked–boiled materials, while non-fermented samples were linked to higher sugar release. PLS regression linked sensory attributes such as umami, nutty, and buttery notes to free amino acids, esters to fermented and sweet odours, and texture attributes such as chewiness to springiness. In extruded faba protein concentrate, fermentation reduced beany, bitter, and astringent notes while producing fruity and fermented flavours. Drying processes partly counteracted these attributes by adding roasted and cereal-like notes and modifying texture, with drum drying producing tougher samples with longer fibrils. In wheat breads with faba bean sourdough, PCA distinguished sourdough from non-sourdough controls, mainly explained by differences in acidity, organic acids, free amino acids, phenolics, and starch digestibility. A second PCA indicated that starch digestibility was strongly associated with sourdough parameters, suggesting opportunities to tailor sourdough fermentation to modulate nutritional outcomes.

Finally, the joint model using INFOGEST data from different fermented food models and their respective unfermented controls showed a strong association of the degree of protein hydrolysis (DH%) with the fermentation induced by two or more strains. This PLS model successfully demonstrated the potential of consortia fermentations, among different fermentation conditions considered, for inducing protein hydrolysis across the developed food models.

Taken together, these predictive models underline the central role of fermentation in steering the nutritional, sensory, and techno-functional properties of cereal–pulse-based foods. By linking process parameters with compositional and sensory outcomes, this deliverable provides a framework for the rational design of plant-based foods that are sustainable, nutritious, and appealing to consumers.

5. Deviations, if applicable

No deviations.

6. References

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7. Annexes

No annexes.