The flavor of faba bean ingredients and extrudates : Chemical and sensory properties

Tuccillo, Fabio

2022-12

Tuccillo , F , Kantanen , K , Wang , Y , Diaz , J M R , Pulkkinen , M , Edelmann , M , Knaapila , A , Jouppila , K , Piironen , V , Lampi , A-M , Sandell , M & Katina , K 2022 , ' The flavor of faba bean ingredients and extrudates : Chemical and sensory properties ' , Food Research International , vol. 162 , 112036 . https://doi.org/10.1016/j.foodres.2022.112036

http://hdl.handle.net/10138/350837
https://doi.org/10.1016/j.foodres.2022.112036

Food Research International 162 (2022) 112036

Contents lists available at ScienceDirect

Food Research International

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

The flavor of faba bean ingredients and extrudates: Chemical and sensory properties

Fabio Tuccillo [a,*], Katja Kantanen [a], Yaqin Wang [a], Jose Martin Ramos Diaz [a], Marjo Pulkkinen [a,b], Minnamari Edelmann [a], Antti Knaapila [a], Kirsi Jouppila [a], Vieno Piironen [a], Anna-Maija Lampi [a], Mari Sandell [a,c], Kati Katina [a]

[a] Department of Food and Nutrition Sciences, P.O. Box 66 (Agnes Sjöbergin katu 2), FI-00014 University of Helsinki, Helsinki, Finland
[b] Finnish Food Authority, Mustialankatu 3, FI-00790 Helsinki, Finland
[c] Functional Foods Forum, University of Turku, FI-20014 Turku, Finland

ARTICLE INFO

Keywords:
Faba bean
Meat alternative
High-moisture extrusion
Flavor precursors
Lipid-degrading enzyme
Off-flavor
Flavor-active compound
Generic descriptive analysis
Chemometrics
Sensometrics

ABSTRACT

Faba bean, processed into ingredients (flour, protein concentrate, protein isolate), can be extruded to meat alternatives with a fibrous texture. Despite its importance for consumer acceptance, not enough is known about the flavor of faba bean ingredients nor about the chemical and sensory changes caused by high-moisture extrusion. Therefore, the aim of this work was to describe the flavor of faba bean ingredients and the corresponding extrudates and to understand how their composition affects the perception of sensory attributes. Firstly, faba bean protein ingredients and extrudates were characterized for lipid-degrading enzymatic activities, flavor precursors, and volatile and non-volatile flavor-active compounds. Secondly, sensory profiling was conducted. Thirdly, partial least squares regression was applied to understand the relationship between chemical and sensory data. This study showed that faba bean protein concentrate had the strongest taste and aftertaste (respectively 7 and 6, on a 0–10 intensity scale), bitterness (6–7), and pea flavor and odor (respectively 6 and 5), whereas faba bean protein isolate had the strongest cereal flavor (4) and odor (4), and off-flavor (2) and off-odor (3). Faba bean flour had the mildest flavor. High-moisture extrusion brought several chemical changes to the ingredients, including the formation of several volatile compounds and inactivation of lipid-degrading enzymes. Only traces of tannins were found in extrudates. The presence of free phenolics, vicine, and convicine was linked to strong taste and aftertaste, bitterness, and a drying sensation of the mouth, whereas lipid oxidation products were related to pea, cereal, and off-odors and flavors.

1. Introduction

Faba bean (*Vicia faba* L.) has gained popularity for environmental, nutritional, and technological reasons (Sharan et al., 2020). Besides having a high protein content, faba bean is a good source of bioactive compounds and minerals (Dhull et al., 2021). The environmental benefits of faba bean cultivation are due to its high protein yield, nitrogen fixation, and ability to grow also in colder climate regions where it could replace imported soybean as a plant protein source (Karkanis et al., 2018; Lizarazo et al., 2015; Flores et al., 2013). Furthermore, cultivation and consumption of faba bean is widely spread in the Mediterranean and Middle Eastern regions (Pasqualone et al., 2020).

Faba bean seeds can be processed into various ingredients, such as flour (FF), protein concentrate (FPC), and protein isolate (FPI) (Sharan et al., 2020), which are potential ingredients with which to produce meat analogues by high-moisture extrusion (HME). In HME, high-moisture content (>40 %), high temperature (100–180 °C), and shear forces are applied to texturize the plant protein ingredients into meat-resembling fibrous structures (Guyony et al., 2022). HME is conducted with a twin-screw extruder with a long cooling die, which plays a crucial role in the formation of fibrous structures.

The potential of faba bean in meat alternative production will only

Abbreviations: FPC, Faba bean protein concentrate; FPI, Faba bean protein isolate; FF, Faba bean flour; HME, High-moisture extrusion; LOX, Lipoxygenase; ASE, Accelerated solvent extraction; TPA, Texture profile analysis; WAC, Water absorption capacity; OAC, Oil absorption capacity; WHC, Water hydration capacity; GDA, Generic descriptive analysis; PCA, Principal component analysis; PLS, Partial least squares regression.

* Corresponding author.
E-mail address: fabio.tuccillo@helsinki.fi (F. Tuccillo).

https://doi.org/10.1016/j.foodres.2022.112036
Received 28 June 2022; Received in revised form 3 October 2022; Accepted 9 October 2022
Available online 14 October 2022

be realized if consumers like and accept its flavor, which is challenging (Roland et al., 2017). Two main aspects that negatively affect the sensory perception of faba bean are its beany flavor and bitter taste. The former is caused by the presence of volatile compounds (e.g., hexanal, 1-hexanol, 2-pentylfuran) generated from auto- and enzymatic oxidation of fatty acids, whereas the latter originates from the binding of several non-volatile compounds (e.g., tannins, phenolics, saponins) to bitter taste receptors. The theoretical base of the present work is presented in the recent review by Wang et al. (2022) on the flavor challenges in extruded pant-based meat alternatives.

Currently, only limited research has been carried out on the flavor of faba bean ingredients and extruded meat alternatives. And, to the best of our knowledge, this is the first study to extensively characterize the sensory profile of faba bean ingredients and extrudates, and to elucidate the chemistry behind it. The focus in the literature has been on the texture of faba bean extrudates, and not much attention has been given to flavor (Do Carmo et al., 2021; Ferawati et al., 2021). Furthermore, by bridging the current knowledge gap, this work lays the foundations for our future research, including a deeper understanding of bitter-causing non-volatile compounds in faba bean, and sustainable bioprocessing methods for flavor improvement.

Given this, the present paper aims at (I) chemically characterizing the lipid-degrading enzymatic activities, flavor precursors, and flavor-active compounds of faba bean ingredients and extrudates, (II) defining their sensory profile, (III) exploring the relations between the study's chemical and sensory data, and (IV) investigating the structural properties of the extrudates and their relation to the sensory attributes of appearance and texture.

2. Materials and methods

2.1. Study materials

Faba bean ingredients consist of four commercially available raw materials (two batches of faba bean protein concentrate, faba bean protein isolate, and faba bean flour) and of four blends made from these raw materials. Name of the samples, their acronyms, origins, and cultivars are reported in Table 1. Faba bean extrudates were produced using a laboratory co-rotating twin-screw extruder (Thermo Prism PTW24 Thermo Haake, Polylab System, Karlsruhe, Germany) equipped with a long cooling die and operating with reverse osmosis water from the following ingredients: FPCa, FPCb, 70 % FPCa (+30 % FPI), 70 % FPCb (+30 % FPI), 40 % FPCa (+30 % FPI + 30 % FF), 40 % FPCb (+30 % FPI + 30 % FF). The ratio of the FPI and FPC mixture (calculated protein content 74 % of solids) was chosen based on the capability to form fibrous structures (Kantanen et al., 2022). The ratios of the other mixtures were chosen to test the fiber formation capability of FPC alone and in the mixture with FPI and FF (calculated protein content 64 % of solids). The total mass feed rate was 50 g/min, and the water content was 60 %. The extruder barrel consisted of six temperature-controlled zones with the following temperature profile: 25, 40, 80, 100, 120, and 150 °C, respectively. The seventh zone had a temperature of 150 °C and the temperature of the long cooling die was set at 80 ℃. The screw speed was set at 500 rpm. After extrusion, samples were manually cut, sealed in plastic bags, and stored at –20 ℃. Samples for chemical analyses were further processed by homogenizing three 50-g pieces, twice, in a Grindomix GM200 homogenizer (Retsch GmbH, Verder Scientific, Haan, Germany) equipped with a knife mill for 10 s at 7,000 rpm. Part of the homogenized samples was also freeze-dried at < 1 mbar for 24 h by using a Christ Alpha 1–2 freeze-dryer (Osterrode am Harz, Germany). Images of the faba bean extrudates are shown in the supplementary material in Fig. 1.

Table 1
Faba bean ingredients: raw materials and blends.

Raw materials	Acronym	Origin	Cultivar
Faba bean protein concentrate (A)*	FPCa	AGT Food and Ingredients, Regina, SK, Canada	Snowbird, Tabasco, Malik, FBP-4
Faba bean protein concentrate (B)*	FPCb	Suomen Viljava OY, Helsinki, Finland	Kontu
Faba bean protein isolate	FPI	AGT Food and Ingredients, Regina, SK, Canada	Snowbird, Tabasco, Malik, FBP-4
Faba bean flour	FF	Suomen Viljava OY, Helsinki, Finland	Kontu
Blends*			
70 % FPCa + 30 % FPI	70 % FPCa		
70 % FPCb + 30 % FPI	70 % FPCb		
40 % FPCa + 30 % FPI + 30 % FF	40 % FPCa		
40 % FPCb + 30 % FPI + 30 % FF	40 % FPCb		

* Samples were processed by means of high-moisture extrusion.

2.2. Chemical analyses

All chemical analyses were conducted on raw materials and homogenized extrudates, with few exceptions. Proximate analyses were conducted on raw materials only, analyses on fats and non-volatile flavor-active compounds were conducted on freeze-dried extrudates, and volatile analysis was conducted also on the blends. The number of replicates (N, 2–4) are shown in the headings of tables and figures in the results section.

2.2.1. Proximate analyses (protein, fat, starch, moisture, and ash)

The protein content (%) was quantified with the Dumas combustion method, using a factor of 6.25 to calculate the protein content. The fat content (%) was measured as the sum of fatty acid methyl esters (see section 2.2.3) and the starch content (%) using a Total starch assay kit (Megazyme ltd., Wicklow, Ireland). Methods AACC 44-15A and AACC 08–01 (AACC Approved Methods of Analysis, 1961, 1974) were used to calculate the moisture (%) and ash contents (%), respectively.

2.2.2. Analysis of lipase and lipoxygenase activities

The method from Yang et al. (2017) was adopted for lipase and lipoxygenase (LOX) assays with some modifications. Briefly, enzymes were extracted with 4 mL of MQ-water from 0.4 g of the raw materials and 0.6 g of the extrudates. Lipase activity was measured with a spectrophotometric method based on hydrolysis of *para*-nitrophenyl butyrate substrate (Sigma-Aldrich, St. Louis, MO, USA). The reaction mixture contained 200 μL of the enzyme extract and 800 μL of a 2 mM substrate solution in a 50 mM potassium phosphate buffer (pH 8) containing 0.1 % Triton X-100. The activity as μmol min^{-1} g^{-1} of the sample was calculated based on the increase in absorption at 405 nm during 150 s using the molar absorptivity of $\varepsilon = 16.05$ $mM^{-1}cm^{-1}$ for *para*-nitrophenol. LOX activity was measured with a spectrophotometric method based on the formation of conjugated dienes of the linoleic acid substrate. Two-hundred μL of the enzyme extract was added to 2.6 mL of the potassium phosphate buffer (pH 6) and 200 μL of a 10 mM linoleic acid solution. Absorbance was measured after 5 min at 234 nm, and the activity as μmol min^{-1} g^{-1} was calculated using the molar absorptivity of $\varepsilon =$ 26,000 $M^{-1}cm^{-1}$ of conjugated dienes.

2.2.3. Analyses of flavor precursors (fatty acids, free fatty acids, free amino acids, and mono-, di-, oligosaccharides)

Fatty acids (%) were determined as their methyl esters after lipid extraction using accelerated solvent extraction (ASE) (Dionex ASE-200, Thermo Fisher Scientific, Sunnyvale, CA, USA). Ethanol was used to extract lipids from 1 g of the samples mixed with Ottawa Sand (P/N S23-3, Fisher Scientific OY, Vantaa, Finland) and placed in 11 mL extraction cells. Extraction conditions were as follows: temperature, 125 ℃;

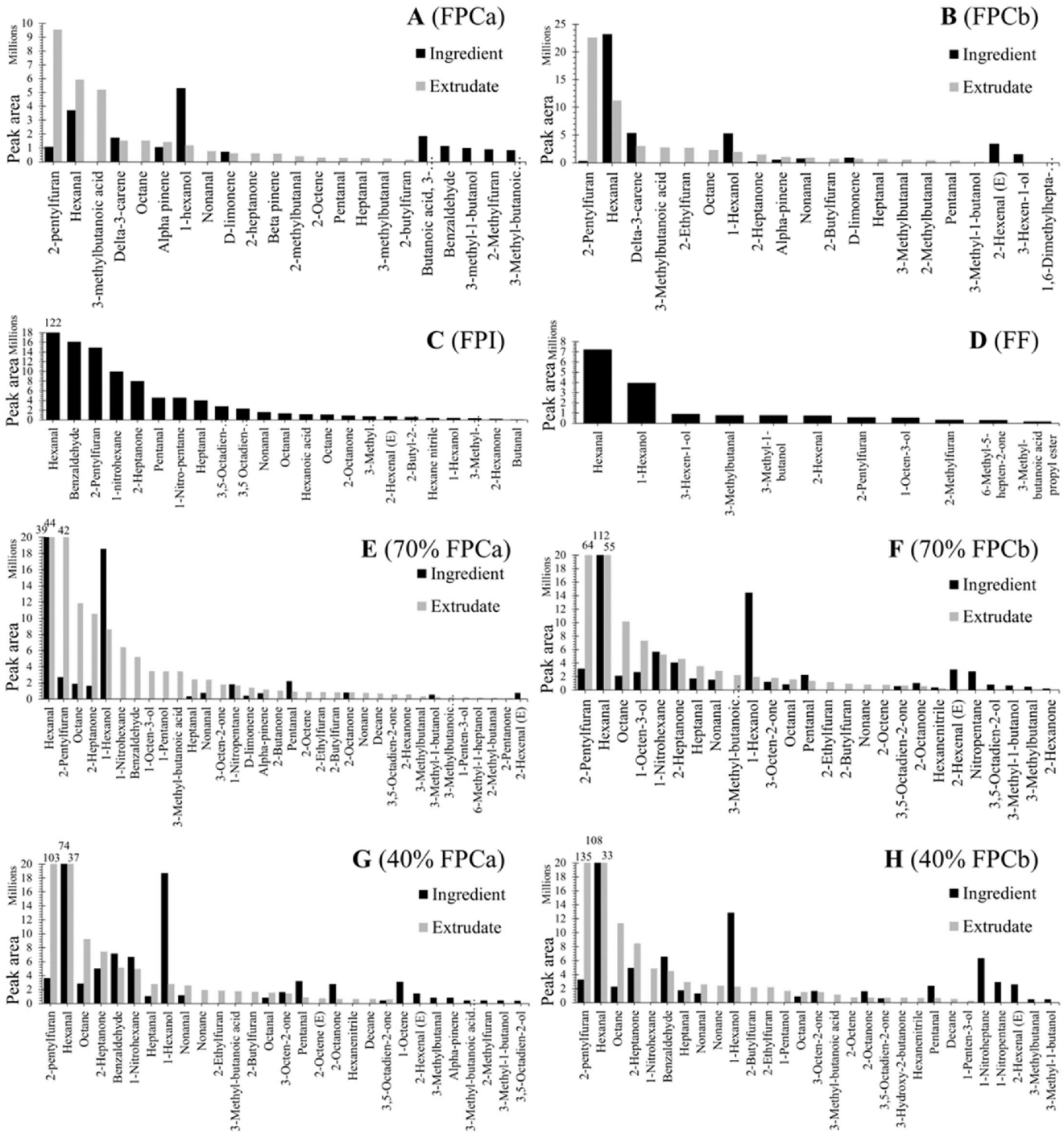


Fig. 1. Volatile profiles of faba bean protein concentrate (FPC) as ingredient and extrudate (A, B), faba bean protein isolate (FPI) (C), faba bean flour (FF) (D), and blends and extrudates made of faba bean ingredients (E, F, G, H). $N = 4$. Coefficients of variation are shown in Supplementary Table 2. Full name of the partially displayed compounds: A) Butanoic acid, 3-methyl-, 3-methylbutyl ester. B) 1,6-Dimethylhepta-1,3,5-triene. C) 3,5-Octadien-2-one; 3,5-Octadiene-2-ol or 3-Octen-2-one; 3-Methyl butanoic acid; 2-Butyl-2-octenal, 3-Methyl-butanal. E) 3-Methylbutanoic acid ethyl ester. F) 3-Methylbutanoic acid. G) 3-Methylbutanoic acid propyl ester.

pressure, 1500 psi; preheat time, 5 min; heat time, 6 min; static time, 11 min; flush volume, 60 %; purge time, 60 *sec*; static cycles, 1. The internal standard, 5 mg of methyl nonadecanoate (Nu-Check-Prep, Elysian, MN, USA) was added to the lipid extracts, which were subjected to methylation using BF_3 in methanol. Fatty acid methyl esters were collected in heptane and analyzed by gas chromatography with flame-ionization detection (GC-FID) as described earlier (Yang et al., 2017). Quantification was carried out using the internal standard method, and fat content was calculated as the sum of fatty acid methyl esters.

Free fatty acids (mg/g) were analyzed as presented by Yang et al. (2019) with a minor modification. After lipid extraction using ASE, free fatty acids were determined by normal-phase high-performance liquid chromatography (NP-HPLC) using evaporative light scattering detection. Separation was achieved using the same diol column as before and with a gradient elution using solvents A (0.1 % of acetic acid in heptane) and B (0.1 % acetic acid, 3 % of 2-propanol in heptane). The gradient

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), 40–50 min (97:3). Free fatty acids were quantified using external standard calibration with oleic acid (Nu-Check-Prep, Elysian, MN, USA).

Free amino acids (mg/g) were extracted from 0.1 g of the raw materials and 0.2 g of the extrudates with 1–2 mL of 70 % ethanol in water by sonication in a water bath for 20 min at room temperature. After centrifugation (10 min, 10,000 rpm), supernatants were collected, and extraction was repeated twice. Extracted soluble proteins were removed by precipitation with 6 M 5-sulfosalicylic acid followed by centrifugation. Volumes of the supernatants were adjusted to 5 mL after pH adjustment to 7–10. Free amino acids were determined using a pre-column derivatization method described in Graʕa et al. (2022) and based on an Acquity UPLC system (Waters AccQ-Tag TM Ultra, Waters, Milford, MA, USA) and the manufacturer's application system guide note (Waters 2007). Chromatographic separation and quantification of amino acids with L-norvaline (Sigma Aldrich, Schnelldorf, Germany) as an internal standard were performed as in Graʕa et al. (2022).

Mono-, di-, and oligosaccharides (mg/g) (glucose, fructose, galactose, sucrose, melibiose, raffinose, stachyose, and verbascose) were analyzed by adapting the method from Xu et al. (2017). Samples of 0.1 g were extracted in a water bath (100 ℃, 10 min) with 5 mL of MQ-water. After centrifugation (10 min, 10,000 × *g*), supernatants (500 μL) were filtered using Amicon® ultra-centrifugal filters with a 10 K molecular cut off (Merck Millipore, Darmstadt, Germany) and injected into a high-performance anion exchange chromatograph with a pulse amperometric detector (Waters, Milford, MA, USA). Identification and quantification of the compounds were reported by Xu et al. (2017).

2.2.4. Analyses of flavor-active compounds (volatiles, total free phenolics, total condensed tannins, soyasaponins B, βG, vicine and convicine)

Volatiles were analyzed by headspace solid-phase micro extraction gas chromatography mass spectrometry (HS-SPME GC–MS) as described in Lampi et al. (2020) with some modifications. Suspensions of 2.0 g of the raw materials with 75 % (w/v) MQ-water or 2.0 g of the extrudates were placed in 20-mL amber SPME vials. For the extraction, a 1 cm (50/30 μm phase thickness) divinylbenzene/carboxen/polydimethylsiloxane fiber (Supelco, Sigma Aldrich, St. Louis, MO, USA) was employed with the following parameters: incubation of 10 min and extraction of 30 min with an agitation speed of 250 rpm and a temperature of 50℃, which was selected based on pre-experiments aimed at investigating the repeatability of results and liberation of desired compounds (e.g. hexanol, hexanal) using different temperatures. GC–MS analysis was done as reported by Yang et al. (2019), using a SPB-624 column (30 m × 0.25 mm i.d., 1.4 μm). Peaks were integrated manually and compounds were identified by comparing their MS spectra to the Wiley 7 N library (Wiley Registry™ of Mass Spectral Data). Results were given as peak areas (counts × s × 10^6) of total ion counts (*m/z* 40–300).

Free phenolic compounds were extracted as described by Li et al. (2008) with modifications and determined with the Folin-Ciocalteu assay. Samples of 0.1 g were extracted three times with 1.5 mL of 80 % aqueous EtOH (v/v) by sonicating in a water bath (10 min) and centrifuging (10 min, 5000 rpm). The supernatants were combined into 5-mL flasks and filled to the exact volume with 80 % EtOH. Three mL of the extracts was evaporated and followed by dissolving the residues in 500 μL of 10 % aqueous MeOH. For spectrophotometric measurement, 200 μL of extracts, 1 mL of Folin-Ciocalteu reagent (1:10; Supelco, Merck Millipore, Darmstadt, Germany), and 800 μL of Na_2CO_3 solution (7.5 %) were combined and followed by incubation for 30 min. Absorbance of samples and gallic acid standard (Sigma Aldrich, Schnelldorf, Germany) solutions was measured at 765 nm. Results were expressed as mg/gallic acid equivalents (GAE)/g sample.

Total condensed tannins were determined according to the vanillin method (Price et al., 1978) modified by Sun et al. (1998). Briefly, 0.2 g of the raw materials (except 0.1 g for FF) and 0.4 g of the extrudates were extracted in 2 mL of 1 % H_2SO_4 in MeOH with shaking at room temperature (90 min, 750 rpm). After centrifuging (10 min, 8,000 rpm), 1.5 mL of vanillin (Sigma Aldrich, Schnelldorf, Germany) solution (1 % in MeOH) and 2.5 mL of 7.2 N H_2SO_4 in MeOH were added to 0.5 mL of the extracts prior to incubation for 15 min at 35 ℃. Spectrophotometric measurement of samples and (+)-catechin standards (Sigma Aldrich, Schnelldorf, Germany) was carried out at 500 nm. Results were expressed as mg catechin equivalents (CE) /g of sample.

For the determination of soyasaponins B and βG (mg/g), 0.1 g of the samples was extracted three times with 1.5 mL of 20 % aqueous MeOH. After incubation on a table shaker (20 min, 300 rpm) and centrifugation (5 min, 4,000 rpm), supernatants were combined and volumes were adjusted to 5 mL. The chromatographic separation of saponins was performed on an Acquity Waters UPLC system (Waters, Milford, MA, USA) equipped with an HSS T3 C18 column (1.8 μm, 2.1 × 150 mm; Waters, Milford, MA, USA) and a photodiode array detector (PDA; 190–600 nm), and using a binary gradient elution system at a flow rate of 0.3 mL/min. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B), each modified with 0.025 % trifluoroacetic acid, and the following mobile phase gradient (A:B) was maintained during a 17 min run: 0–1 min (95:5); 1–8 min (50:50); 8–10 min (50:50); 10–12 (20:80); 12–13 min (20:80); 13–14 min (95:5). The autosampler was maintained at 4 °C, the column was operated at 30 °C, and an injection volume of 30 μL was used. Soyasaponin B was identified based on retention time and the UV spectrum (maximum at 200 nm) of the standard (Phytolab GmbH & Co. KG; Vestenbergsgreuth, Germany). Soyasaponin βG was identified based on its absorption maximum at 294 nm (Hu et al., 2002). A six-point calibration curve (concentration ranger 5–10 μg/mL) at 200 nm was created for soyasaponin Bb and used to calculate concentrations of both saponins.

Vicine and convicine (mg/g) were analyzed by HPLC. 4.5 mL of Milli-Q water was used to extract vicine and convicine from 0.1 g-samples with added 1.6 mg uridine (Sigma Aldrich, St. Louis, MO, USA) used as the internal standard. After vortexing the mixtures and letting them stand for 15 min, they were centrifuged (10 min, 9,600 g) and the supernatants were collected and boiled for 5 min. The supernatants were analyzed by HPLC with PDA (at 273 nm) and the results were calculated as presented earlier (Pulkkinen et al., 2015).

2.3. Physical and mechanical properties

2.3.1. Texture profile, cutting strength, and color analyses

A Texture Analyser (Stable Micro Systems, Godalming, Surrey, England) was used to conduct the Texture profile analysis (TPA) and the cutting strength on the extrudates according to Kantanen et al. (2022) with some modifications. For the TPA, partially frozen extrudates were cut in a cubical shape with dimensions of 24 mm (width) × 24 mm (length) × 14 mm (height) and thawed in an oven for 30 min at 40 °C. Hardness, gumminess, springiness, and chewiness were calculated from the force-distance curve. For the cutting strength, partially frozen extrudates were cut to small pieces with dimensions of 20 mm (width) × 30 mm (length) × 10 mm (height) and thawed as in TPA. The cutting strength was measured from perpendicular and longitudinal directions to the flow of the material inside the long cooling die and determined as the maximum peak force from the force-distance curve. The color of the extrudates was measured in ten replicates using a Minolta Chroma Meter CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan). The color was expressed as CIE-lab parameters as L* (lightness), a* (- greenness, + redness), and b* (- blueness, + yellowness).

2.3.2. Water and oil absorption capacity and water hydration capacity

The water absorption capacity (WAC) and oil absorption capacity (OAC) of the extrudates were analyzed as by Lin et al. (2002) with some modifications. For preparation, the extrudates were thawed for 30 min at room temperature and cut into pieces with dimensions of 20 mm (width) × 30 mm (length) × 10 mm (height) and then placed at –70 °C

for 24 h. The samples were then freeze dried for 3 days using a Lyovac GT 2 freeze-dryer (Amsco Finn-Aqua GmbH, Hürth, Germany) at pressure < 0.5 mbar. After being freeze dried, the samples were rehydrated with either 40 mL of deionized water or rapeseed oil in a 50-mL falcon tube and placed in a water bath at 50 ℃ for 16 h. The water and oil absorption capacities were calculated according to Lin et al. (2002). For the analysis of water hydration capacity (WHC), the extrudates were thawed at room temperature for 1 h, homogenized using a blender, freeze dried, and milled using an ultra-centrifugal mill (Retsch ZM 200, Haan, Germany) at 10,000 rpm with a 0.5 mm sieve. The WHC was analyzed according to the AACC Method 56–30 (AACC Approved Methods of Analysis, 1978).

2.4. Sensory evaluation

2.4.1. Participants

Ten (of which eight were females) and 13 (of which 10 were females) participants were trained for the evaluation of faba bean ingredients and extrudates, respectively. They were recruited by e-mail within the Food and Nutrition Department mailing list of the University of Helsinki, and selected based on previous experience in sensory profiling. Participants were informed about the study and consented to participate by a signed written consent. Taking part in the study was voluntary, but participation in all training and evaluation sessions was highly advised. The study was conducted in accordance with the Declaration of Helsinki for experiments involving humans and ethical principles of sensory research at our department, reviewed by the University of Helsinki Ethical Review Board in Humanities and Social and Behavioral Sciences (Statement 15/2020).

2.4.2. Evaluated faba bean samples

Faba bean ingredients were evaluated as suspensions in 75 % (w/v) tap water. The mixture was vigorously mixed with a spoon for a couple of minutes, until no lumps were visible. Ten grams of the mixture was served in a plastic cup covered with a lid. Frozen extrudates were cut into pieces of 4.5 × 1.5 × 1 cm and placed in covered plastic cups. Each cup, containing three pieces, was placed in the oven (30 min at 40 ℃) for thawing. Ingredients and extrudates were served at room temperature (~20 ℃).

2.4.3. Training and evaluation procedures

Generic descriptive analysis (GDA) was carried out in June 2021 (for the extrudates) and in September 2021 (for the ingredients) in the sensory laboratory, conforming to ISO 8589. Three and four training sessions (1–2 h/session) were held for the sensory profiling of the ingredients and the extrudates, respectively. Attributes for the ingredients were adapted from previous work carried out in the department and modified by the panel when needed. Descriptors for the extrudates were generated entirely by the panel. Assessors evaluated the proposed reference samples and found a collective agreement on their description and intensity. Table 2 shows the final profiles, having 13 attributes (four for odor, three for flavor, and six for taste and mouthfeel) for the ingredients and 20 attributes for the extrudates (three for appearance, five for odor, five for texture, three for flavor, and four for taste and mouthfeel). English and Finnish were equally used throughout the training and evaluation sessions.

Samples were evaluated three times, after one complete training session. This included the whole sample set and allowed the panel to familiarize themselves with the conditions of the formal sensory sessions. Each session included all samples (eight for the ingredients, six for the extrudates) that were served with a three-digit code label, in a randomized block design among assessors and sessions. Panelists were asked to recognize and evaluate the intensity of each attribute in the samples by comparing it to the reference sample. They were instructed to refer to the description and intensity of the reference samples prior to each evaluation. Intensity scores were rated on a line scale (from 0 = "not at all" to 10 = "very strong"). Panelists were asked to drink water and chew a puffed corn snack, while taking a small break between samples to clean their palate. Data collection was carried out with RedJade© (RedJade Sensory Solutions LLC, Boulder, CO, USA) for the

Table 2
List of sensory attributes, descriptions, reference samples, and their intensity used for the sensory profiling of ingredients and extrudates.

Attribute (Ingredients)	Description	Reference	Intensity
Total odor intensity	Intensity of the whole odor experience	n/a	n/a
Pea odor	Raw pea, pea pods	Overnight soaked (dry) peas	5
Off-odor	Unpleasant odor, oxidized fat, musty	n/a	n/a
Cereal odor	Cereal, hay	Semi wet oat flakes	8
Pea flavor	Raw pea, pea pods	Same as "Pea odor"	9
Off-flavor	Unpleasant odor, oxidized fat, musty	n/a	n/a
Cereal flavor	Cereal, hay	Same as "Cereal odor" (ingredients)	9
Total taste intensity	Intensity of the whole taste experience	n/a	n/a
Umami	Meaty, savory, cooked protein products	0.1 % Monosodium glutamate	6
Sweetness	Sweet, sugar in water	2 % Sucrose	6
Bitterness	Bitter, sharp sensation, need for sweetness	0.7 % Caffeine	10
Dryness of the mouth	Drying, puckering sensation, need for hydration	Paper tissue	9
Total aftertaste intensity	Intensity of the remaining taste after swallowing	n/a	n/a
Attribute (Extrudates)			
Layered appearance	Clarity of the layers, noticeable edges	Half-cut onion	9
Matte appearance	Opposite of shiny, difficult to reflect light	Eraser	10
Fresh yeast appearance	Surface of a slice of a fresh yeast cube	Slice of a yeast cube	10
Total odor intensity	Intensity of the whole odor experience	n/a	n/a
Cooked pea odor	Boiled, cooked peas	Boiled peas	8–9
Cereal odor	Cereal, hay	Oat oil	9
Grass odor	Freshly cut grass, green leaves	Alpha-alpha sprouts	7
Yeast odor	Yeast, yeast-caused food spoilage	Same as "Fresh yeast appearance"	10
Gummy texture	Difficult to break, elastic, bounce back	Gummy candy	10
Flaky texture	Surface breaks easily in form of flakes	Freshly baked croissant	8
Fibrous texture	Separation into fibers, sinewy, muscle tissue	Pulled oats	8
Floury texture	Small powdery granules	Wheat flour	10
Soft texture	Opposite of hard, easy to chew	White bread	9–10
Veggie stock flavor	Vegetable stock, broth, mixture of vegetables	Commercial vegetable broth	8–9
Cooked pea flavor	Boiled, cooked peas	Same as "Cooked pea odor"	9–10
Cereal flavor	Cereal, hay	Same as "Cereal odor" (extrudates)	8–9
Total taste intensity	Intensity of the whole taste experience	n/a	n/a
Umami	Meaty, savory, cooked protein products	0.5 % Monosodium glutamate	8
Bitterness	Bitter, sharp sensation, need for sweetness	0.7 % Caffeine	9
Dryness of the mouth	Drying, puckering sensation, need for hydration	Paper tissue	9–10

n/a = not applicable (no reference was used).

GDA of the ingredients and FIZZ © (Version 2.51, Biosystèmes, Couternon, France) for the GDA of the extrudates.

2.5. Statistical analyses

Results were expressed as mean values of replicate measurements. Apart from Tables 5 and 6 (and Supplementary Table 3), standard deviations or coefficients of variation of all measurements were reported separately for readability reasons (Supplementary Tables 1 and 2). IBM SPSS® Statistics (Version 28, IBM®, Chicago, IL, USA) was used for descriptive and univariate analyses, whereas The Unscrambler® X (Version 10.5, Aspen Technology Inc, Bedford, MA, USA) was used for multivariate analysis. Inferential analyses were set with $\alpha = 0.05$ as the threshold for statistical significance.

Panel performance was evaluated by conducting a three-way analysis of variance (ANOVA) on the attribute scoring data of 10 Participants*3 Replicates*8 Samples and of 13 Participants*3 Replicates*6 Samples for ingredients and extrudates, respectively. The variable Samples was set as fixed factors, whereas Participants and Replicates were considered as random factors. Main effects and two-way interactions were observed. The quality of the sensory data was further assessed by looking at the distribution of the scoring data for ingredients (240 observations = 10 panelists*3 replicates*8 samples) and extrudates (234 observations = 13 panelists*3 replicates*6 samples) for each sensory attribute. We considered the following indicators of normality of distribution: skewness and kurtosis (values between −1 and 1) and the Kolmogorov-Smirnova and Shapiro-Wilk tests (no significance). The Kolmogorov-Smirnova test was conducted using the Lilliefors significance correction. Homogeneity was checked with Levene's test for each attribute, considering the observations of all participants given for all samples during the three sensory sessions. When cases of inhomogeneity were found, the Welch test of equality of means was conducted instead of ANOVA. However, no differences were observed in detecting type I or type II errors between the two tests. To check the repeatability of the single assessors and the panel's agreement on the attributes' meaning, we conducted principal component analysis (PCA) on the panelists' scores from each session for each attribute. Correlation loadings of the first two components close to each other indicated good repeatability and agreement. Singular Value Decomposition (SVD) was used as the algorithm for PCA, which was validated using a full cross validation method. To characterize the sensory profile, ANOVA models were applied separately to ingredients and extrudates for each attribute. When a statistically significant difference was found among samples, Tukey's HSD post-hoc test was applied. Finally, three separate models were made using partial least squares regression (PLS) analysis. The first accounted for faba bean raw materials (blends were not included), and it had the chemical measurements as predictors (X-variables) and sensory attributes as responses (Y-variables). The second model was carried out as the first but had as samples only faba bean extrudates. The third model considered the physico-mechanical properties and the protein, fat, starch, and moisture contents of the extrudates (predictors) with their appearance and textural properties (responses). All data was auto scaled and mean-centered. Full cross validation and the Kernel algorithm were applied.

3. Results

3.1. Chemical characterization

The chemical characterization of faba bean ingredients and extrudates is shown in Table 3. Among the ingredients, FPI had the highest protein content and the lowest starch content. The opposite was found for FF, whose fat content was the smallest. The largest fat fraction was observed in FPI and FPCa. The highest lipid-degrading enzymatic activity was found in FPCa, followed by FPCb, and FF. The most abundant fatty acids were (in descending order) linoleic, oleic, and palmitic acids. Small amounts of α-linolenic were found. Similar levels of fatty acids were observed in all ingredients, except that higher contents of linoleic acid were found in FPI and FPCa than in FF and FPCb. The largest content of total free fatty acids was observed in FPCb, followed by FPI, FF, and FPCa. The lowest levels of free amino acids were found in FPI. With regard to the individual amino acids (Supplementary Table 2), several differences were noted among the ingredients. Briefly, arginine was the most abundant amino acid in all samples except in FPI, which was richer in leucine. Mono-, di-, and oligosaccharides were not detected in FPI, sucrose was the highest in FF, and verbascose was the highest in both FPC.

HME caused the loss of lipase and LOX activities and an increase in free amino acids. The measured levels of several amino acids (e.g., arginine, glycine, histidine, lysine, and tyrosine) were higher after extrusion, but smaller or stable in the case of other amino acids (e.g., leucine, glutamic acid, and tyrosine).

Table 3
Chemical composition (protein, fat, starch—including resistant, moisture, ash contents), enzymatic activities (lipase, lipoxygenase—LOX), fatty acid profile, free amino acids, di- and oligosaccharides (sucrose, stachyose, melibiose, verbascose) of faba bean ingredients (Ing) and extrudates (Ex). $N = 3$ ($N = 2$ for enzymatic activities, $N = 4$ for di- and oligosaccharides).

Composition	FPCa		FPCb		FPI	FF	70 % FPCa	70 % FPCb	40 % FPCa	40 % FPCb
	Ing	Ex	Ing	Ex	Ing	Ing	Ex	Ex	Ex	Ex
Protein (% dm)	64.4	n/a	63.9	n/a	96.0	31.9	n/a	n/a	n/a	n/a
Fat (% dm)	4.6	3.7	3.2	3.7	4.8	2.5	3.8	3.7	3.9	3.8
Starch (% dm)	6.3	n/a	8.0	n/a	0.3	36.1	n/a	n/a	n/a	n/a
Moisture (% fw)	6.3	54.4	9.5	57.2	7.3	11.0	56.7	57.7	57.1	58.0
Ash (% dm)	6.6	n/a	6.4	n/a	6.2	3.6	n/a	n/a	n/a	n/a
Lipase (μmol/g/min dm)	8.6	n/d	7.3	n/d	n/d	6.9	n/d	n/d	n/d	n/d
LOX (μmol/g/min dm)	178.3	n/d	148.7	n/d	n/d	140.6	n/d	n/d	n/d	n/d
Palmitic acid, 16:0 (% dm)	0.6	0.5	0.5	0.5	0.8	0.3	0.6	0.6	0.7	0.7
Oleic acid, 18:1 (% dm)	1.0	0.9	0.9	1.0	1.3	0.5	1.1	1.1	1.1	1.1
Linoleic acid, 18:2 (% dm)	2.6	1.8	1.7	1.8	2.2	1.4	1.8	1.7	1.9	1.8
α-Linolenic acid,18:3 (% dm)	0.2	0.1	0.1	0.1	0.1	0.1	0.1	<0.1	0.1	0.1
Free fatty acids (mg/g dm)	0.3	0.3	2.0	1.7	1.5	0.5	0.7	2.0	0.5	1.2
Free amino acids (mg/g dm)	3.0	4.1	7.8	8.9	0.6	5.6	3.2	6.6	3.2	5.6
Sucrose (mg/g dm)	1.9	1.9	1.1	2.0	n/d	3.5	1.5	1.4	1.6	1.6
Stachyose (mg/g dm)	1.3	1.9	0.8	1.4	n/d	0.9	1.4	1.2	1.0	0.9
Melibiose (mg/g dm)	0.6	0.5	n/d	0.8	n/d	0.7	0.5	0.6	0.3	0.4
Verbascose (mg/g dm)	6.2	6.7	3.7	7.0	n/d	4.2	5.1	4.7	4.0	3.7

FPC = faba bean protein concentrate, FPI = faba bean protein isolate, FF = faba bean flour, dm = dry matter, fw = fresh weight, n/a = not applicable (analysis was not conducted), n/d = not detected. Standard deviations are shown in Supplementary Table 1.

3.2. Volatile compounds

In characterization of possible flavor-active volatiles in faba bean ingredients and extrudates, several classes of compounds were detected among the samples, including alcohols, aldehydes, alkanes, alkenes, aromatic compounds, esters, furans, ketones, organic acids, nitro compounds, and terpenes (Fig. 1). Most compounds detected in the ingredients were aldehydes and alcohols. Hexanal, 1-hexanol, and 2-pentylfuran were found in all ingredients. Hexanal's and 2-pentylfuran's peak areas were the highest in FPI. Moreover, FPI had the richest volatile profile compared to the other raw materials, in terms of compounds detected and their peak areas. Volatile profiles were raw material dependent, as all materials had several compounds that were found in one specific ingredient only. For instance, terpenes (alpha-pinene, delta-3-carene, D-limonene) were detected only in the concentrates. Moreover, differences in the two batches of FPC were found. However, when FPC was used in blends with FPI and FF, less differences were observed.

Overall, HME brought an increase in the number of volatile compounds, despite the loss of some. Also, differences in peak areas were observed between ingredients and extrudates. The most drastic increase was noted for 2-pentylfuran, while a decrease in 1-hexanol was noted. 3-Methyl-butanoic acid, 2- and 3- methylbutanal, pentanal, heptanal, and 2-butylfuran were generated from the extrusion of FPCa and FPCb, among other compounds specific to the two ingredients. However, when compared to the respective raw material, less differences were observed in the extrudates made with the different batches of FPC. Interestingly, no typical Maillard reaction products (e.g., pyrazines, alkylpyrazines, alkylpyridines, or furanones) were detected in the extrudates.

3.3. Non-volatile compounds

Fig. 2 shows the concentration of free phenolics, total condensed tannins, soyasaponins B and βG, vicine, and convicine in raw materials and extrudates. The highest concentration of free phenolics was observed in FPCa, followed by FPCb, and FF. Negligible contents were observed in FPI. In the extrudates the contents were smaller than in the raw materials. Regarding tannins, the highest concentration was measured in FF, approximately double of that which was quantified in FPC and FPI. Only traces of condensed tannins were found in the extrudates by the used total tannin assay. Soyasaponin B was detected only in one ingredient (FPI) and two extrudates. Soyasaponin βG, on the other hand, was detected in all raw materials, except in FPI. The highest concentration was observed in FPCb, followed by FF, and FPCa. Slightly smaller contents were found in the extrudates than in the respective raw material. Vicine content was the highest in FPCa, followed by FPCb and FF, whereas concentration of convicine was the highest in FPCb, followed by FPCa and FF. Contents of both were negligible in FPI. Extrudates had higher measured vicine content compared to the ingredients; however, levels of convicine were similar.

3.4. Physical and mechanical properties of the extrudates

The results from the physical and mechanical properties of the extrudates are shown in Table 4. Extrudates containing 70 % FPC had the lowest hardness and cutting strengths. The highest cutting strengths were measured for extrudates containing 40 % FPC. Regarding the different batches of FPC, extrudates containing FPCb had higher values for mechanical properties (hardness, chewiness, springiness, and

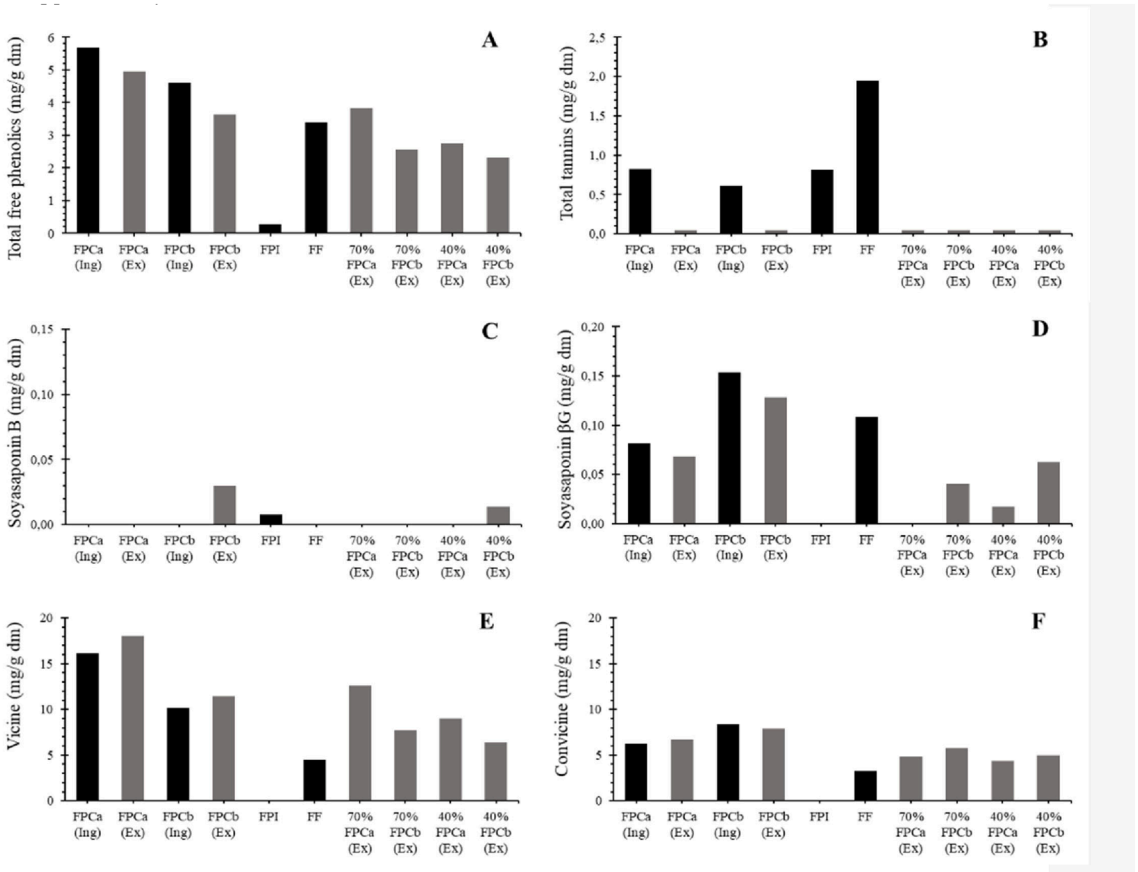


Fig. 2. Concentration (mg/g) of total free phenolics (A), total tannins (B), soyasaponin B (C), soyasaponin βG (D), vicine (E), and convicine (F) in the ingredients (Ing) faba bean protein concentrate (FPC), faba bean protein isolate (FPI), and faba bean flour (FF), and in the extrudates (Ex). Black bars are used for the raw materials, gray bars for the extrudates. $N = 3$. Standard deviations are shown in Supplementary Table 1.

Table 4
Mechanical and physical properties of faba bean extrudates.

Physico-mechanical properties	FPCa	FPCb	70 % FPCa	70 % FPCb	40 % FPCa	40 % FPCb
Cutting strength, longitudinal	7.09	7.13	4.84	6.34	9.45	9.15
Cutting strength, perpendicular	5.35	4.61	4.25	4.53	5.88	6.5
Gumminess	87.02	93.85	88.39	103.96	100.07	136.45
Springiness	0.78	0.81	0.85	0.89	0.82	0.8
Chewiness	67.33	75.37	75.45	92.38	81.83	108.98
Hardness	237.72	240.82	189	208.01	218.49	266.9
L*	57.25	50.59	61.48	51.25	52.1	50.53
a*	−0.61	3.08	−0.82	1.39	−0.46	0.69
b*	26.96	23.13	24.51	18.44	15.03	14.42
Water absorption capacity	179.95	170.22	165.22	162.35	181.77	173.78
Oil absorption capacity	71.4	80.17	84.03	76.5	85.8	77.38
Water hydration capacity	1.95	2.23	2.28	2.18	2.18	2.23

FPC = faba bean protein concentrate. Standard deviations are shown in Supplementary Table 1.

gumminess) compared to extrudates with a corresponding content of FPCa. No such clear differences were observed in cutting strengths between the extrudates containing different contents of either FPCa or FPCb. However, differences between longitudinal and perpendicular cutting strengths were shown. Longitudinal cutting strength was higher compared to perpendicular cutting strength in all extrudates. The results from the color measurements showed that extrudates with FPCa were lighter, less red, and more yellow compared to extrudates with a corresponding content of FPCb. The lowest WAC was measured for extrudates with 70 % FPC. Additionally, WAC was higher in extrudates containing FPCa compared to extrudates with a corresponding content of FPCb.

3.5. Sensory profiles

Panelists evaluated in three sessions the intensity of key attributes used to describe faba bean ingredients and extrudates. Panel performance was assessed by carrying out a three-way ANOVA (Supplementary Table 4) and showed the ability of the assessors to distinguish among samples (ingredients, significance found for 10/13 attributes; extrudates, 15/20 attributes). Moreover, as typical in sensory studies, three-way ANOVA showed significance for the factor Participant for all attributes and for the interaction Sample*Participant for some attributes (ingredients, 8/13 attributes; extrudates, 18/20 attributes). A significant effect of Replicate was observed for only one attribute (extrudates: fresh yeast appearance), while the interactions Sample*Replicate was found significant for only a few attributes (ingredients, 5/13 attributes; extrudates, 5/20 attributes). Similarly, the interaction Participant*Replicate showed only a few cases of significance (ingredients, 5/13 attributes; extrudates, 9/20 attributes). As commonly accepted in datasets of sensory data, both the Kolmogorov-Smirnova and Shapiro-Wilk tests showed a departure from normality, apart from a few attributes (Supplementary Table 5). However, skewness and kurtosis indicated a normal distribution (values ranging from −1 to 1) for all attributes, except for off-odor and off-flavor in the ingredients and for yeast odor in the extrudates. Moreover, kurtosis was only outside the range for the following attributes used to describe extrudates: fresh yeast appearance, cereal odor, floury texture, and cereal flavor. Among the attributes of the two sensory profiles, panelists showed great agreement and repeatability in evaluating the following: pea and cooked pea odor and flavor, cereal odor and flavor (for the ingredients), taste intensity, bitterness, and total aftertaste intensity (for the ingredients) (Supplementary Fig. 2 and Fig. 3).

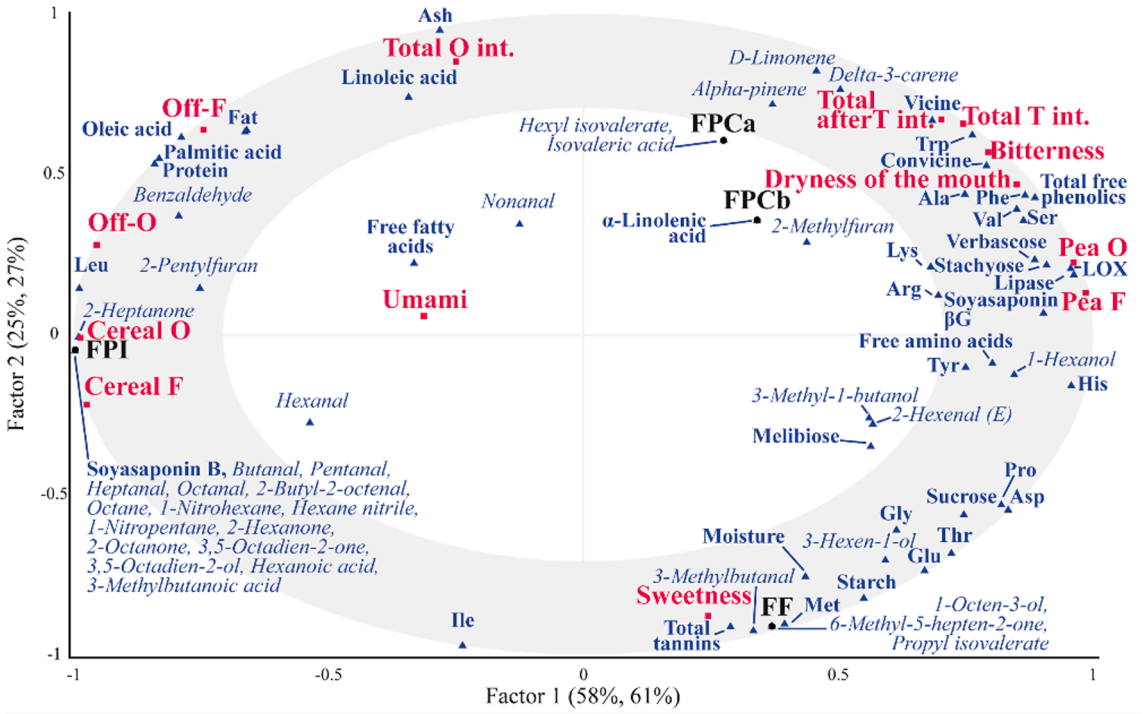
The sensory profiles of faba bean ingredients and extrudates are reported in Table 5 and Table 6, respectively. Several differences were observed among the ingredients. FPC was characterized by high pea odor, pea flavor, and bitter taste. This resulted in a strong taste and aftertaste. Conversely, FPI and FF had the mildest taste and aftertaste. The latter was slightly more bitter than FPI, which, on the other hand, had the strongest cereal aroma and flavor. The sensory characteristics of the blends logically reflect the ones of the raw materials at the given contents. No statistically significant differences were observed for total odor intensity, off-flavor, umami, sweetness, and dryness of the mouth. Extrudates differed also for several attributes. In the ones where flour was added (40 % FPC), a more layered and less fresh yeast-like appearance was found, compared to the ones where only FPI was added (70 % FPC), or to the ones containing only FPC. Extrudates made entirely by FPCa had higher odor intensity and cooked pea aroma and flavor than did the other samples. Interestingly, the sensory properties of the two batches of FPC did not differ significantly as ingredients. Furthermore, the two FPCs differed from the rest of the samples for having stronger bitterness and total taste intensity.

3.6. Role of chemical composition on flavor

Regression analysis was applied to understand the relationships between the chemical composition of faba bean ingredients and extrudates and their flavor. Therefore, these results are intended to be an indicative tool to reveal relationships of enzymatic activity, flavor precursors, and flavor-active compounds on the perceived flavor properties.

In the PLS model for the ingredients (Fig. 3), 83 % of the variation in chemical data explained 88 % of the variation in the sensory data. FPI had a negative correlation loading on Factor 1, along with aroma and flavor attributes related to cereal and off-flavors and odors. These seemed to be related to the presence of several volatile compounds, including octanal, pentanal, heptanal, butanal, 1-nitrohexane, 3-methyl butanoic acid, 2-octanone, 3,5-octadien-2-one, 3,5-octadien-2-ol, 1-nitropentane, hexanoic acid, hexane nitrile, 2-butyl-2-octenal, 2-hexanone, 2-heptanone, 2-pentylfuran, benzaldehyde, and to a lesser extent hexanal and nonanal. Relations between the attribute off-flavor and oleic acid and total fat content were observed, as well as between odor intensity and linoleic acid. A slight link between umami and the presence of nonanal and free fatty acids was noted. FPC and FF had a positive correlation loading with Factor 1 but had respectively a positive and negative correlation loading with Factor 2. Close to FPC are the correlation loadings indicating strong taste and aftertaste intensity, bitterness, dryness, and pea odor and flavor, while FF is correlated with sweetness. Strong taste, bitterness, and dryness of the mouth had a correlation with the presence of certain amino acids (e.g., tryptophan, phenylalanine, valine), free phenolics, vicine, and convicine. Soyasaponin βG contributed only slightly to those sensory qualities, whereas Soyasaponin B did not at all. Lipid-degrading enzymatic activity had a role in the perception of pea odor and flavor, which are correlated with the presence of 1-hexanol. Sweetness in FF seems to be related with higher starch and moisture content, and with the presence of sucrose and melibiose.

In the PLS model for the extrudates (Fig. 4), 65 % of the variation in chemical data explained 80 % of the variation in the sensory data. Extrudates made only of FPC had a positive correlation loading on Factor 1, but they located further from each other compared to the respective ingredients. This indicated that HME had a slightly different effect on these two materials, as one extrudate (made from FPCa) had stronger bitterness, cooked pea flavor and odor, odor and taste intensity, and drying quality compared to the other (made from FPCb). As for the ingredients, bitterness and drying mouthfeel seemed to be related to the presence of free phenolics, vicine, convicine, and several amino acids



Abbreviations: Int. = intensity, His = histidine, Ser = serine, Arg = arginine, Gly = glycine, Asp = asparagine, Glu = glutamate, Thr = threonine, Ala = alanine, Pro = proline, Lys = lysine, Tyr = tyrosine, Met = methionine, Val = valine, Ile = isoleucine, Leu = leucine, Phe = Phenylalanine, Trp = tryptophan.

Fig. 3. Partial least squares regression (PLS) plot of the interaction between sensory attributes (responses, squares, in red) and flavor-related chemical components (predictors, triangles, in blue) of the following faba bean ingredients (circles, in black): two batches of faba bean protein contrate (FPCa, FPCb), faba bean protein isolate (FPI), and faba bean flour (FF). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 5
Sensory attributes, F values (ANOVA), significance (ANOVA), and averages intensities (standard deviation) of faba bean ingredients. $N = 30$.

Attribute	F	Sig	FPCa	FPCb	FPI	FF	70 % FPCa	70 % FPCb	40 % FPCa	40 % FPCb
Total odor intensity	1.9	ns	5.4 (1.7)	4.5 (1.8)	4.8 (2.2)	4 (2.1)	4.1 (1.6)	4.2 (1.9)	4.2 (1.9)	4.2 (1.3)
Pea odor	16.8	***	4.9 (1.9) c	4.6 (1.6) c	1.6 (1.3) a	4.2 (2) c	2.2 (1.4) ab	2.9 (1.8) b	2.8 (1.5) ab	2.3 (1.5) ab
Off-odor[a]	6.2	***	1.3 (1.7) ab	0.8 (1.3) a	2.8 (2.8) b	0.4 (0.8) a	1.3 (1.5) ab	1.9 (2.3) ab	1.6 (2.2) ab	1.8 (2.2) ab
Cereal odor[a]	12.8	***	1 (1.1) a	1.4 (1.4) ab	4 (2.3) d	1 (1.2) a	3.4 (2.1) bcd	2.6 (2) cd	2.5 (1.8) bc	2.7 (2.1) bcd
Pea flavor	13.7	***	6.3 (2.1) d	6.2 (2.3) d	2.1 (1.9) a	5.9 (2.1) cd	4.1 (2.2) b	4.6 (2.3) bc	4.8 (1.7) bcd	4.3 (1.7) bc
Off-flavor	0.8	ns	1.8 (2.2)	1.9 (2.4)	2.4 (2.9)	1.1 (1.5)	1.6 (1.9)	2 (2.3)	1.7 (1.7)	1.8 (1.9)
Cereal flavor	5.6	***	1.4 (1.4) a	1.5 (1.6) a	3.9 (2.2) c	1.9 (1.9) ab	2.9 (2.1) abc	2.3 (2.1) ab	3 (2.2) abc	3.1 (2.2) bc
Total taste intensity	8.5	***	6.9 (1.8) b	6.8 (1.7) b	4.3 (2.1) a	5.1 (1.9) a	5.4 (1.6) a	5.1 (2.2) a	4.7 (1.7) a	4.8 (1.2) a
Umami	0.1	ns	2.2 (1.6)	1.9 (1.6)	2.2 (2)	2.1 (1.5)	2 (1.5)	2 (1.6)	2.2 (1.8)	2.1 (1.5)
Sweetness	2.0	ns	1.3 (1.1)	1 (0.9)	1.2 (1.3)	2 (1.3)	1.3 (1.1)	1.3 (1.1)	1.6 (1.2)	1.5 (0.9)
Bitterness	14.8	***	5.9 (2.4) cd	6.6 (2) d	2.1 (1.9) a	3.8 (2.2) b	4.5 (1.8) bc	3.7 (2.4) b	3.4 (2) ab	3.4 (1.8) ab
Dryness of the mouth	1.6	ns	4.1 (2)	4.3 (1.9)	3.2 (2.2)	3.7 (2)	3.8 (1.6)	4.1 (1.9)	3.1 (1.7)	3.5 (1.7)
Total aftertaste intensity	8.9	***	5.6 (2.2) bc	6.1 (2.4) c	2.9 (1.8) a	3.7 (2) a	4.3 (1.9) ab	4.1 (2.2) ab	3.5 (2) a	3.5 (1.4) a

[a] Due to inhomogeneous variance, ANOVA's F values and significance are replaced by Welch test's statistics. Sig = statistical significance, FPC = faba bean protein concentrate, FPI = faba bean protein isolate, FF = faba bean flour, ns = no significance, *** = $p \leq 0.001$. Different letters in each row indicate statistically significant differences (Tukey's HSD).

(phenylalanine, tryptophan, and histidine). Contrary to what was observed for the ingredients, 3-methyl butanoic acid correlated more with cooked pea flavor and aroma than with the cereal and unpleasant qualities. Moreover, the cooked pea flavor and aroma seemed to be linked as well to the presence of alpha-pinene, nonanal, and D-limonene. The attributes of odor and taste intensity were related to the presence of 2- and 3-methylbutanal. Correlation loadings of the extrudates made from blends of faba bean ingredients were found to be located on the negative side of Factor 1, where correlation loadings of the attributes cereal odor and flavor are also located. There, oleic and linoleic acid were closely located. The extrudates made of 70 % FPC and 30 % FPI were found to be located on the negative side of Factor 2, whereas the ones made of 40 % FPC, 30 % FPI, and 30 % FF were found to be located on the positive side. The former seemed to be more closely linked to a veggie stock flavor compared to the other samples. This sensory quality was linked to the presence of benzaldehyde, decane, and heptanal. Extrudates containing FF seemed to have a stronger umami taste, whose correlation loadings were closely linked to those of glutamic acid, starch, 2-pentylfuran, and 3-hydroxy-2-butanone.

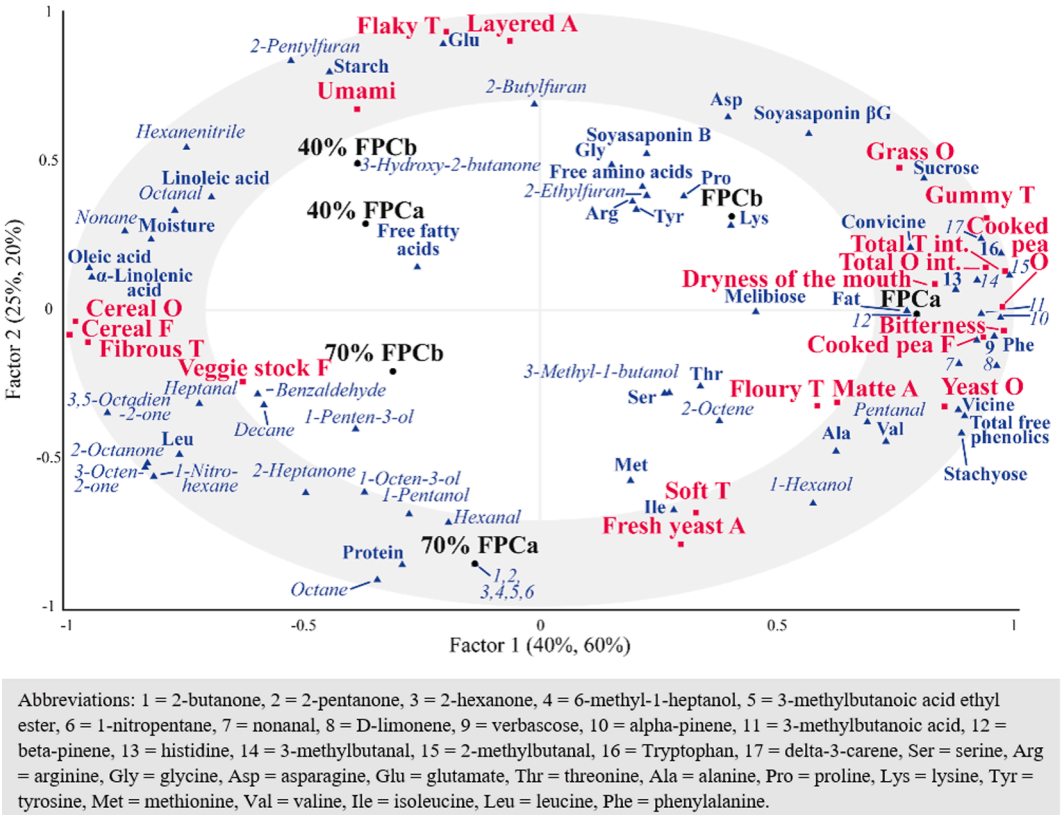
3.7. Role of physical and mechanical properties and proximate composition on appearance and texture

Regression analysis was conducted to study the effects of physical and mechanical properties, and proximate composition on the textural attributes of the extrudates evaluated in the sensory analysis. The

Table 6
Sensory attributes, F values (ANOVA), significance (ANOVA), and averages intensities (standard deviation) of faba bean extrudates. $N = 39$.

Attribute	F	Sig	FPCa	FPCb	70 % FPCa	70 % FPCb	40 % FPCa	40 % FPCb
Layered appearance[a]	22.3	***	6.1 (2) b	6.1 (2.8) b	3.7 (2.4) a	4.4 (2.5) a	7.6 (1.5) c	7.5 (1.9) bc
Matte appearance	7.3	***	7.4 (1.7) b	7.1 (2) b	6.4 (2.2) ab	7.4 (1.6) b	6.2 (2.1) ab	5.2 (2.2) a
Fresh yeast appearance	12.2	***	5.1 (2.8) b	5.4 (2.6) b	6.5 (2.2) b	6.1 (2.4) b	3 (2.8) a	3.3 (2.9) a
Total odor intensity	12.1	***	7 (1.7) b	5.5 (1.6) a	4.4 (1.8) a	4.5 (1.9) a	4.6 (1.8) a	4.6 (2) a
Cooked pea odor	14.3	***	7.2 (1.9) c	5.6 (2) b	4.6 (2) ab	4 (2) a	4.2 (2.2) ab	4 (2.3) a
Cereal odor	2.2	ns	2.8 (1.9)	3.3 (2.3)	3.8 (2.4)	4.2 (2.2)	4.1 (2.5)	4 (2.4)
Grass odor	0.8	ns	2.7 (2)	2.6 (2.1)	2.1 (2)	1.9 (1.7)	2.4 (2.3)	2.3 (1.9)
Yeast odor	1.7	ns	3.1 (2.8)	2.1 (2.3)	2.3 (2.2)	1.9 (2.1)	1.9 (2.3)	1.7 (2.1)
Gummy texture[a]	0.7	ns	5.5 (2.6)	5.3 (2.5)	4.8 (1.8)	4.7 (2.2)	4.9 (2)	4.9 (2.2)
Flaky texture	3.9	**	6.4 (2.2) ab	6.9 (2) ab	5.7 (2.6) a	6.1 (2.2) ab	7.4 (2) b	7.4 (2) b
Fibrous texture	4.2	***	4 (2.1) a	4.5 (2.2) ab	5.3 (2.2) ab	5.9 (2.1) b	5.3 (2.3) ab	5.5 (2.1) b
Floury texture	1.9	ns	5.2 (2.2)	5 (2)	5.2 (2.5)	4.2 (2.4)	5 (2.5)	4.1 (2.3)
Soft texture	1.6	ns	4.4 (1.6)	3.9 (1.7)	4.6 (1.8)	3.9 (1.7)	4.2 (1.7)	3.7 (1.7)
Veggie stock flavor	0.3	ns	2.3 (2.2)	2.5 (2.2)	2.5 (2)	2.8 (2.1)	2.4 (1.7)	2.5 (1.9)
Cooked pea flavor	13.3	***	7.4 (1.7) c	5.5 (2.4) b	5.1 (2.1) ab	4 (2.1) a	4.7 (2.3) ab	4.1 (2.3) a
Cereal flavor	1.7	ns	2.6 (2.1)	3 (2.2)	3.6 (2.2)	3.7 (2.2)	3.7 (2.4)	3.7 (2.4)
Total taste intensity	12.8	***	7.7 (1.6) b	6.9 (1.8) b	5.5 (2.1) a	5 (2) a	5.4 (1.9) a	5.2 (2) a
Umami	1.2	ns	3.1 (1.9)	3 (2.2)	2.7 (1.9)	3.2 (1.9)	3.8 (2.3)	3.4 (2)
Bitterness	15.0	***	7.7 (2.1) b	7.2 (2.4) b	5.6 (2.5) a	4.7 (2.5) a	4.4 (2.2) a	4.5 (2.3) a
Dryness of the mouth	1.8	ns	5.5 (2.6)	5.7 (2.6)	5 (2.7)	4.4 (2.5)	4.3 (2.5)	4.9 (2.8)

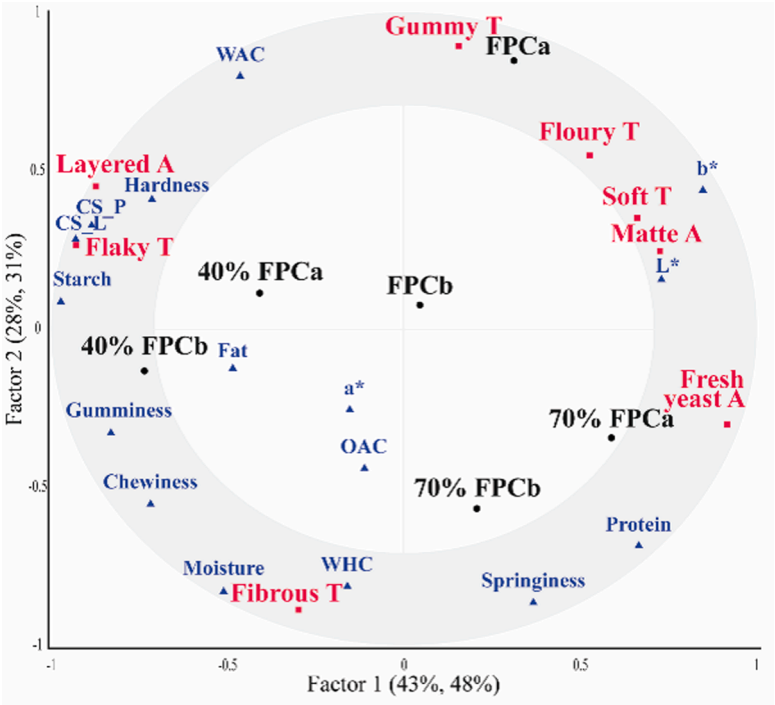
[a] Due to inhomogeneous variance, ANOVA's F values and significance are replaced by Welch test's statistics. Sig = statistical significance, FPC = faba bean protein concentrate, ns = no significance, ** = $p \leq 0.01$ *** = $p \leq 0.001$. Different letters in each row indicate statistically significant differences (Tukey's HSD).



Abbreviations: 1 = 2-butanone, 2 = 2-pentanone, 3 = 2-hexanone, 4 = 6-methyl-1-heptanol, 5 = 3-methylbutanoic acid ethyl ester, 6 = 1-nitropentane, 7 = nonanal, 8 = D-limonene, 9 = verbascose, 10 = alpha-pinene, 11 = 3-methylbutanoic acid, 12 = beta-pinene, 13 = histidine, 14 = 3-methylbutanal, 15 = 2-methylbutanal, 16 = Tryptophan, 17 = delta-3-carene, Ser = serine, Arg = arginine, Gly = glycine, Asp = asparagine, Glu = glutamate, Thr = threonine, Ala = alanine, Pro = proline, Lys = lysine, Tyr = tyrosine, Met = methionine, Val = valine, Ile = isoleucine, Leu = leucine, Phe = phenylalanine.

Fig. 4. Partial least squares regression (PLS) plot of the interaction between sensory attributes (responses, squares, in red) and flavor-related chemical components (predictors, triangles, in blue) of six extrudates (circles, in black) made of 100 % faba bean protein concentrate (FPCa, FPCb), 70 % faba bean protein concentrate and 30 % faba bean protein isolate (70 % FPCa, 70 % FPCb), and 40 % faba bean protein concentrate, 30 % faba bean protein isolate, and 30 % faba bean flour (40 % FPCa, 40 % FPCb). A = Appearance, F = Flavor, O = Odor, T = Texture. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

correlation loadings plot from the PLS model (Fig. 5) shows that 71 % of the variation in the physical and mechanical properties and proximate composition explained 79 % of the variation in sensory data. Cutting strengths, hardness, starch content, gumminess, and chewiness positively correlated with layered appearance and flaky texture and negatively correlated with fresh yeast and matte appearance and with a soft and floury texture. Protein content negatively correlated with layered appearance and flaky texture but positively correlated with fibrous texture. Moreover, moisture content and WHC positively correlated with fibrous texture. The lightness and yellow color positively correlated with matte and fresh yeast appearance. Additionally, extrudates containing 40 % FPC were associated with a layered appearance and flaky texture,



Abbreviations: CS_L = longitudinal cutting strength, CS_P = perpendicular cutting strength, WAC = water absorption capacity, OAC = oil absorption capacity, WHC = water hydration capacity.

Fig. 5. Partial least squares regression (PLS) plot of the interaction between sensory attributes (responses, squares, in red) of appearance (A), texture (T), and physical and mechanical properties and proximate composition (protein, fat, starch, moisture) (predictors, triangles, in blue) of six extrudates (circles, in black) made of 100% faba bean protein concentrate (FPCa, FPCb), 70% faba bean protein concentrate and 30% faba bean protein isolate (70% FPCa, 70% FPCb), and 40% faba bean protein concentrate, 30% faba bean protein isolate, and 30% faba bean flour (40% FPCa, 40% FPCb). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

whereas extrudates containing 100 % and 70 % of FPCa were more associated with a soft and floury texture.

4. Discussion

Current research has been focusing extensively on the potential of plant proteins to be processed into meat alternatives, considering their nutritional and environmental benefits. A common limitation that studies and review articles have found is the beany flavor and bitterness of pulses (Roland et al., 2017). With regard to faba bean, this has been theoretically discussed and not fully studied. Recent studies on the flavor of pulse ingredients and extrudates have focused on soy, and lacked the use of multivariate statistical methods between sensory and physicochemical data (Wang et al., 2022). Because of this, there is no clear understanding on possible chemical compounds connected to off-flavors. The present study explored the possible links between sensory attributes and flavor precursors, meant as food components that can react to produced possible flavor-active compounds under certain conditions, and flavor-active compounds, meant as molecules that at varying thresholds can be perceived as aroma, taste, or both (Wang et al., 2022).

Our study showed that the flavor of faba bean is raw material- and processing-dependent. The strong taste, bitterness, and mouth-drying sensation of FPC clearly related to the presence of free phenolics, which have been previously described as being highly flavor-active molecules (Heiniö et al., 2016), which can have different bitterness and astringency thresholds (Huang & Xu, 2021). Therefore, further investigation is needed to understand which individual free phenolic compounds are linked to bitterness in faba bean. Vicine and convicine, which also contributed to bitterness, have been mainly researched for their anti-nutritional activity (Khazaei et al., 2019) and not for their contribution to flavor. Many non-volatile compounds have been indicated as bitter-causing factors in pulses (Campos-Vega et al., 2010), but our findings indicated that saponins and condensed tannins in faba bean play only a minor role. However, genetic variability and processing of the seeds (e.g., whether dehulling is done as in the production of the ingredients of this study) can affect the level of tannins and other anti-nutrients (Crépon et al., 2010; Egounlety & Aworh, 2003) and thus their role in flavor. Moreover, our results support findings that soyasaponin βG, confirmed to be more bitter than soyasaponin B (Heng et al., 2006), was converted to soyasaponin B during protein isolation (Lin et al., 2006). The pea-like odor and flavor of FPC and the cereal and off-odors and flavors of FPI were attributed to the presence of lipid oxidation products, which can be formed in significant levels in materials with very low fat content (Murat et al., 2013). Interestingly, our research revealed that pea odor and flavor are linked with enzymatic reactions, as confirmed by the presence of 1-hexanol, which is formed in the LOX pathway (Lampi et al., 2020). Differently, several autoxidation products (e.g., hexanoic acid, heptanal, octanal, and nonanal) seemed to have a relationship with the cereal and off-odors and flavors of FPI. The mechanism of lipid-derived off-flavors formation in pulses was described in detail by Wang et al. (2022). As example, nonanal and octanal are formed from the scission of the bond closer to the carboxylate function of 10- and 11-hydroperoxides of oleic acid, respectively. Hexanal, on the other hand, is formed from 13-hydroperoxide of linoleic acid. Our results confirmed that auto- and enzymatic oxidation release different volatile compounds, hence distinctively influencing the flavor profile of the food matrix. Therefore, it is crucial to use an appropriate sensory lexicon for describing the “beaniness” of pulses (Chigwedere et al., 2022). Among the investigated ingredients, faba bean flour had the lowest protein content, the mildest flavor, and a slight sweet taste. Some studies showed that proteins play a key role as carriers of flavor compounds (Guichard, 2002), and that protein-rich ingredients are the most probable for retaining off-flavors (Zhang et al. 2021). We believe that compounds related to off-flavors are likely to concentrate in the protein fraction during air classification and that processing parameters

(e.g., heat treatment) when fractionating and isolating proteins influence the final composition of the ingredient.

The type of ingredient also affected the physical and mechanical properties of the extrudates, which additionally were also affected by the batch of the FPC. The presence of starch in the FF seemed to promote a layered and flaky texture, which could be due to the phase separation to protein- and carbohydrate-rich phases (Ubbink et al., 2022). The addition of polysaccharides to enhance the fibrous structures of meat analogues has been widely studied (Chen et al., 2021; Zhang et al., 2020; Palanisamy et al., 2018). Interestingly, an increased starch content resulted in harder extrudates, whereas an increased protein content resulted in softer extrudates. An increased protein content has earlier been associated with increased hardness, possibly due to increased cross-linking (Zhang et al., 2018). Recent studies showed that it is possible to produce meat alternatives with fibrous structures from faba bean protein concentrate and isolate by means of high-moisture extrusion (Do Carmo et al., 2021; Ferawati et al., 2021; Kantanen et al., 2022). Our study confirmed this and additionally proved that faba bean flour mixed with protein concentrate and isolate can also be extruded to produce fibrous structures. Despite the advantage of its mild flavor, faba bean flour cannot be extruded by itself, since the higher protein content is needed for the formation of fibrous structures (Zhang et al., 2022).

The flavor of the extrudates was highly related to the properties of the ingredients, indicating that the design of raw material mixture is fundamental for the development of appealing meat alternatives. As for the ingredients, free phenolic compounds, vicine, and convicine were the main compounds linked to the bitterness of the extrudates. Condensed tannins were observed only at trace levels, possibly because of the altering of molecular structure and binding to proteins and carbohydrates during extrusion (Duguma et al., 2021; Patterson et al., 2017; Adamidou et al., 2011; Dlamini et al. 2009). The lower levels of tannins did not seem to influence the overall sensory profile of the extrudates. Nevertheless, this study increased the understanding of the flavor-related changes occurring during high-moisture extrusion, which has been overshadowed by the more studied dry extrusion (Wang et al., 2022). So far, the sensory profile of meat alternatives of different protein sources has been described in general terms, referring to the raw materials (e.g., legume, cereal, beany) (De Angelis et al., 2020; Kaleda et al., 2020; Katayama and Wilson, 2008). By comparing ingredients and extrudates, we were able to differentiate the pea flavor and odor (found in the ingredients) from the cooked pea flavor and odor (found in the extrudates). Similarly, the veggie stock flavor indicates the result of a cooking process. Numerous volatile compounds were formed during HME, despite the loss of some. In dry extrusion, this was explained by the Maillard reaction (Kaleda et al., 2020), whereas in extrusion at a lower temperature and at a higher moisture content thermal degradation of lipids is the most probable cause (Wang et al., 2022). Our results are in line with the latter explanation, because ingredients had trace levels of reducing sugars, no decrease in the content of reducing sugar and free amino acids was observed, and no Maillard reaction compounds were detected in the extrudates. However, occurrence of the Maillard reaction during HME of faba bean ingredients cannot be entirely excluded, as it is highly dependent on extrusion conditions (Riha III and Ho, 1996). For instance, Maillard reaction is highly probable to occur in low moisture environments (Leonard et al., 2020), such as in dry extrusion. To the best of our knowledge, no occurrence of the Maillard reaction has been previously documented during HME of faba bean ingredients. Only one study (Do Carmo et al., 2021) indicated that Maillard reaction was the cause of the color changes caused by HME, but formation of volatile compounds was not investigated. In our study, on the other hand, the decrease of free phenolics content during HME suggested that color changes were rather caused by the degradation of phenolic compounds (Nasar-Abbas et al., 2009) than by Maillard reaction.

5. Conclusion

This paper describes the flavor of faba bean ingredients and extrudates by considering their chemical composition and sensory characteristics. Faba bean protein concentrate had strong taste and aftertaste, bitterness, and caused drying of the mouth. This related to the presence of free phenolic compounds, vicine, and convicine. Furthermore, faba bean protein concentrate had strong pea-like qualities. Products from lipid oxidation were linked to the cereal and off-odors and flavors of faba bean protein isolate, whereas faba bean flour had the mildest flavor, despite being rich in tannins. Chemically, high-moisture extrusion caused the inactivation of lipid-degrading enzymes and the release of several volatile compounds, but not products of Maillard reaction. Moreover, only trace levels of condensed tannins were observed in the extrudates. High-moisture extrusion caused several changes in terms of flavor, notwithstanding the fact that the type of ingredient was the main variable responsible for the flavor of the extrudates. Flavor changes included the release of cooked pea odor and flavor, grass and yeast odor, and veggie stock flavor.

CRediT authorship contribution statement

Fabio Tuccillo: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Katja Kantanen:** Investigation, Formal analysis, Methodology, Writing – original draft. **Yaqin Wang:** Investigation, Formal analysis, Methodology, Writing – review & editing. **Jose Martin Ramos Diaz:** Investigation, Methodology. **Marjo Pulkkinen:** Formal analysis, Methodology. **Minnamari Edelmann:** Investigation, Formal analysis, Methodology, Writing – review & editing. **Antti Knaapila:** Conceptualization, Writing – review & editing. **Kirsi Jouppila:** Funding acquisition, Supervision, Validation, Writing – review & editing. **Vieno Piironen:** Conceptualization, Supervision, Validation, Writing – review & editing. **Anna-Maija Lampi:** Conceptualization, Supervision, Validation, Writing – review & editing. **Mari Sandell:** Conceptualization, Supervision, Validation, Writing – review & editing. **Kati Katina:** Conceptualization, Supervision, Funding acquisition, Project administration, 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.

Acknowledgements

This work was financed by the Leg4Life project (Legumes for sustainable food system and healthy life), supported by the Strategic Research Council at the academy of Finland (grant number 327698) and by the EXPRO project (Globally competitive processing technologies for meat alternatives), and supported by the Innovaatiorahoituskeskus Business Finland (funding number 6901/31/2019).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.112036.

References

AACC Approved Methods of Analysis. (1961, revised 1999). Method 44-15. *Approved Methods of Analysis. Association* (11th ed.). Cereals and Grains Association.

AACC Approved Methods of Analysis. (1974, revised 2020). Method 08-01. *Approved Methods of Analysis. Association* (11th ed.). Cereals and Grains Association.

AACC Approved Methods of Analysis. (1978, revised 1999). Method 56-30. *Approved Methods of Analysis. Association* (11th ed.). Cereals and Grains Association.

Adamidou, S., Nengas, I., Grigorakis, K., Nikolopoulou, D., & Jauncey, K. (2011). Chemical composition and antinutritional factors of field peas (Pisum sativum), chickpeas (Cicer arietinum), and faba beans (Vicia faba) as affected by extrusion preconditioning and drying temperatures. *Cereal Chemistry*, *88*(1), 80-86.Campos-Vega, R., Loarca-Piña, G., & Oomah, B. D. (2010). Minor components of pulses and their potential impact on human health. *Food Research International*, *43*(2), 461-482.

Campos-Vega, R., Loarca-Piña, G., & Oomah, B. D. (2010). Minor components of pulses and their potential impact on human health. *Food Research International, 43*(2), 461–482. https://doi.org/10.1016/j.foodres.2009.09.004

Chen, Q., Zhang, J., Zhang, Y., Meng, S., & Wang, Q. (2021). Rheological properties of pea protein isolate-amylose/amylopectin mixtures and the application in the high-moisture extruded meat substitutes. *Food Hydrocolloids, 117*, Article 106732.

Chigwedere, C. M., Wanasundara, J. P., & Shand, P. J. (2022). Sensory descriptors for pulses and pulse-derived ingredients: Toward a standardized lexicon and sensory wheel. *Comprehensive Reviews in Food Science and Food Safety, 21*(2), 999–1023.

Crépon, K., Marget, P., Peyronnet, C., Carrouee, B., Arese, P., & Duc, G. (2010). Nutritional value of faba bean (Vicia faba L.) seeds for feed and food. *Field Crops Research, 115*(3), 329–339.

De Angelis, D., Kaleda, A., Pasqualone, A., Vaikma, H., Tamm, M., Tammik, M. L., … Summo, C. (2020). Physicochemical and sensorial evaluation of meat analogues produced from dry-fractionated pea and oat proteins. *Foods, 9*(12), 1754.

Dhull, S. B., Kidwai, M. K., Noor, R., Chawla, P., & Rose, P. K. (2021). A review of nutritional profile and processing of faba bean (Vicia faba L.). *Legume. Science, e129*.

Dlamini, N. R., Dykes, L., Rooney, L. W., Waniska, R. D., & Taylor, J. R. (2009). Condensed tannins in traditional wet-cooked and modern extrusion-cooked sorghum porridges. *Cereal Chemistry, 86*(2), 191–196.

Do Carmo, C. S., Knutsen, S. H., Malizia, G., Dessev, T., Geny, A., Zobel, H., … Sahlstrøm, S. (2021). Meat analogues from a faba bean concentrate can be generated by high moisture extrusion. *Future Foods, 3*, Article 100014.

Duguma, H. T., Forsido, S. F., Belachew, T., & Hensel, O. (2021). Changes in Anti-nutritional Factors and Functional Properties of Extruded Composite Flour. *Frontiers in Sustainable Food Systems, 5*, 71370.

Egounlety, M., & Aworh, O. C. (2003). Effect of soaking, dehulling, cooking and fermentation with Rhizopus oligosporus on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (Glycine max Merr.), cowpea (Vigna unguiculata L. *Walp) and groundbean (Macrotyloma geocarpa Harms)*. *Journal of food engineering, 56*(2–3), 249–254.

Ferawati, F., Zahari, I., Barman, M., Hefni, M., Ahlström, C., Witthöft, C., & Östbring, K. (2021). High-moisture meat analogues produced from yellow pea and faba bean protein isolates/concentrate: Effect of raw material composition and extrusion parameters on texture properties. *Foods, 10*(4), 843.

Flores, F., Hybl, M., Knudsen, J. C., Marget, P., Muel, F., Nadal, S., … Rubiales, D. (2013). Adaptation of spring faba bean types across European climates. *Field Crops Research, 145*, 1–9.

Graça, C., Edelmann, M., Raymundo, A., Sousa, I., Coda, R., Sontag-Strohm, T., & Huang, X. (2022). Yoghurt as a starter in sourdough fermentation to improve the technological and functional properties of sourdough-wheat bread. *Journal of Functional Foods, 88*, Article 104877.

Guichard, E. (2002). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Reviews International, 18*(1), 49–70.

Guyony, V., Fayolle, F., & Jury, V. (2022). High moisture extrusion of vegetable proteins for making fibrous meat analogs: A review. *Food Reviews International,Volume ahead of print,* 1-26.

Heiniö, R. L., Noort, M. W. J., Katina, K., Alam, S. A., Sozer, N., De Kock, H. L., … Poutanen, K. (2016). Sensory characteristics of wholegrain and bran-rich cereal foods–a review. *Trends in Food Science & Technology, 47*, 25–38.

Heng, L., Vincken, J. P., Hoppe, K., Van Koningsveld, G. A., Decroos, K., Gruppen, H., … Voragen, A. G. J. (2006). Stability of pea DDMP saponin and the mechanism of its decomposition. *Food Chemistry, 99*(2), 326–334.

Hu, J., Lee, S. O., Hendrich, S., & Murphy, P. A. (2002). Quantification of the group B soyasaponins by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry, 50*(9), 2587–2594.

Huang, R., & Xu, C. (2021). An overview of the perception and mitigation of astringency associated with phenolic compounds. *Comprehensive Reviews in Food Science and Food Safety, 20*(1), 1036–1074.

Kaleda, A., Talvistu, K., Tamm, M., Viirma, M., Rosend, J., Tanilas, K., … Tammik, M. L. (2020). Impact of fermentation and phytase treatment of pea-oat protein blend on physicochemical, sensory, and nutritional properties of extruded meat analogs. *Foods, 9*(8), 1059.

Kantanen, K., Oksanen, A., Edelmann, M., Suhonen, H., Sontag-Strohm, T., Piironen, V., … Jouppila, K. (2022). Physical properties of extrudates with fibrous structures made of faba bean protein ingredients using high moisture extrusion. *Foods, 11*(9), 1280.

Karkanis, A., Ntatsi, G., Lepse, L., Fernández, J. A., Vågen, I. M., Rewald, B., ... & Savvas, D. (2018). Faba bean cultivation–revealing novel managing practices for more sustainable and competitive European cropping systems. *Frontiers in plant science*, 1115.

Katayama, M., & Wilson, L. A. (2008). Utilization of soybeans and their components through the development of textured soy protein foods. *Journal of food science, 73*(3), S158–S164.

Khazaei, H., Purves, R. W., Hughes, J., Link, W., O'Sullivan, D. M., Schulman, A. H., … Stoddard, F. L. (2019). Eliminating vicine and convicine, the main anti-nutritional factors restricting faba bean usage. *Trends in Food Science & Technology, 91*, 549–556.

Lampi, A. M., Yang, Z., Mustonen, O., & Piironen, V. (2020). Potential of faba bean lipase and lipoxygenase to promote formation of volatile lipid oxidation products in food models. *Food Chemistry, 311*, Article 125982.

Leonard, W., Zhang, P., Ying, D., & Fang, Z. (2020). Application of extrusion technology in plant food processing byproducts: An overview. *Comprehensive Reviews in Food Science and Food Safety, 19*(1), 218–246.

Li, L., Shewry, P. R., & Ward, J. L. (2008). Phenolic acids in wheat varieties in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry, 56*(21), 9732–9739.

Lin, S., Huff, H. E., & Hsieh, F. (2002). Extrusion process parameters, sensory characteristics, and structural properties of a high moisture soy protein meat analog. *Journal of Food Science, 67*(3), 1066–1072.

Lin, J., Krishnan, P. G., & Wang, C. (2006). Retention of isoflavones and saponins during the processing of soy protein isolates. *Journal of the American Oil Chemists' Society, 83*(1), 59–63.

Lizarazo, C. I., Lampi, A. M., Liu, J., Sontag-Strohm, T., Piironen, V., & Stoddard, F. L. (2015). Nutritive quality and protein production from grain legumes in a boreal climate. *Journal of the Science of Food and Agriculture, 95*(10), 2053–2064.

Murat, C., Bard, M. H., Dhalleine, C., & Cayot, N. (2013). Characterisation of odour active compounds along extraction process from pea flour to pea protein extract. *Food Research International, 53*(1), 31–41.

Nasar-Abbas, S. M., Siddique, K. H. M., Plummer, J. A., White, P. F., Harris, D., Dods, K., & D'antuono, M. (2009). Faba bean (Vicia faba L.) seeds darken rapidly and phenolic content falls when stored at higher temperature, moisture and light intensity. *LWT-Food. Science and Technology, 42*(10), 1703–1711.

Palanisamy, M., Töpfl, S., Aganovic, K., & Berger, R. G. (2018). Influence of iota carrageenan addition on the properties of soya protein meat analogues. *LWT –. Food Science and Technology, 87*, 546–552.

Pasqualone, A., Abdallah, A., & Summo, C. (2020). Symbolic meaning and use of broad beans in traditional foods of the Mediterranean Basin and the Middle East. *Journal of Ethnic Foods, 7*(1), 1–13.

Patterson, C. A., Curran, J., & Der, T. (2017). Effect of processing on antinutrient compounds in pulses. *Cereal Chemistry, 94*(1), 2–10.

Price, M. L., Van Scoyoc, S., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry, 26*(5), 1214–1218.

Pulkkinen, M., Gautam, M., Lampi, A. M., Ollilainen, V., Stoddard, F., Sontag-Strohm, T., … Piironen, V. (2015). Determination of vicine and convicine from faba bean with an optimized high-performance liquid chromatographic method. *Food Research International, 76*, 168–177.

Riha, W. E., III, & Ho, C. T. (1996). Formation of flavors during extrusion cooking. *Food Reviews International, 12*(3), 351–373.

Roland, W. S., Pouvreau, L., Curran, J., van de Velde, F., & de Kok, P. M. (2017). Flavor aspects of pulse ingredients. *Cereal Chemistry, 94*(1), 58–65.

Sharan, S., Zanghelini, G., Zotzel, J., Bonerz, D., Aschoff, J., Saint-Eve, A., & Maillard, M. N. (2020). Fava bean (Vicia faba L.) for food applications: From seed to ingredient processing and its effect on functional properties, antinutritional factors, flavor, and color. *Comprehensive Reviews in Food Science and Food Safety, 20*(1), 401–428.

Sun, B., Ricardo-da-Silva, J. M., & Spranger, I. (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. *Journal of Agricultural and Food Chemistry, 46*(10), 4267–4274.

Ubbink, J., & Muhialdin, B. (2022). Protein physical state in meat analogue processing. *Current Opinion in Food Science, 45*, Article 100822.

Wang, Y., Tuccillo, F., Lampi, A. M., Knaapila, A., Pulkkinen, M., Kariluoto, S., … Katina, K. (2022). Flavor challenges in extruded plant-based meat alternatives: A review. *Comprehensive Reviews in Food Science and Food Safety.*, 1–32.

Waters 2007. UPLC amino acid analysis solution. *System guide 71500129702/Revision B.* Waters Corporation.

Xu, Y., Wang, Y., Coda, R., Säde, E., Tuomainen, P., Tenkanen, M., & Katina, K. (2017). In situ synthesis of exopolysaccharides by Leuconostoc spp. and Weissella spp. and their rheological impacts in fava bean flour. *International Journal of Food Microbiology, 248*, 63–71.

Yang, Z., Piironen, V., & Lampi, A. M. (2017). Lipid-modifying enzymes in oat and faba bean. *Food Research International, 100*, 335–343.

Yang, Z., Piironen, V., & Lampi, A. M. (2019). Epoxy and hydroxy fatty acids as non-volatile lipid oxidation products in oat. *Food Chemistry, 295*, 82–93.

Zhang, J., Liu, L., Zhu, S., & Wang, Q. (2018). Texturisation behaviour of peanut–soy bean/wheat protein mixtures during high moisture extrusion cooking. *International Journal of Food Science and Technology, 53*, 2535–2541.

Zhang, J., Liu, L., Jiang, Y., Shah, F., Xu, Y., & Wang, Q. (2020). High-moisture extrusion of peanut protein-/carrageenan/sodium alginate/wheat starch mixtures: Effect of different exogenous polysaccharides on the process forming a fibrous structure. *Food Hydrocolloids, 99*, Article 105311.

Zhang, J., Kang, D., Zhang, W., & Lorenzo, J. M. (2021). Recent advantage of interactions of protein-flavor in foods: Perspective of theoretical models, protein properties and extrinsic factors. *Trends in Food Science & Technology, 111*, 405–425.

Zhang, Z., Zhang, L., He, S., Li, X., Jin, R., Liu, Q., Chen, S., & Sun, H. (2022). High-moisture extrusion technology application in the processing of textured plant protein meat analogues: A review. *Foods Review International*, 1–36.