DOCTORAL THESIS

Physicochemical, Techno-Functional, and Sensory Properties of Plant Protein Food Ingredients and Their Technological Modification

Kadi Jakobson

TALLINNA TEHNIKAÜLIKOOL
TALLINN UNIVERSITY OF TECHNOLOGY
TALLINN 2026

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41/2026

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TAL TECH PRESS

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This dissertation was accepted for the defence of the degree Doctor of Philosophy in Food Science on 03/06/2026

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Defence of the thesis: 30/06/2026, Tallinn

Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree.

Kadi Jakobson

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ISSN 2585-6898 (paperback)
ISBN 978-9916-80-518-3 (paperback)
ISSN 2585-6901 (PDF)
ISBN 978-9916-80-517-6 (PDF)
https://doi.org/10.23658/taltech.41/2026

Jakobson, K. (2026). *Physicochemical, Techno-Functional, and Sensory Properties of Plant Protein Food Ingredients and Their Technological Modification* [TalTech Press]. https://doi.org/10.23658/taltech.41/2026

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List of publications

The list of author's publications, based on which the thesis has been prepared:

I **Jakobson, K**., Kaleda, A., Adra, K., Tammik, M.-L., Vaikma, H., Kriščiunaite, T., & Vilu, R. (2023). Techno-functional and sensory characterization of commercial plant protein powders. *Foods, 12*(14), 2805. doi.org/10.3390/foods12142805

II **Jakobson, K**., Kaleda, A., Vaikma, H., Sats, A., Rosenvald, S., & Kriščiunaite, T. (2026). Particle size and surface morphology of plant protein powders determine the sensory perception of graininess in liquid matrices. *Applied Food Research*, *6*(1), 101699. doi.org/10.1016/j.afres.2026.101699

III Kaleda, A., Sharma, N., **Jakobson, K**., Stulova, I., & Rosenvald, S. (2025). Fermentation by lactic acid bacteria during pea protein isolation reduces undesirable flavors and changes techno-functional properties. *Food Chemistry*, 492(Part 1), 145380. doi.org/10.1016/j.foodchem.2025.145380

IV Latrofa, V., Kaleda, A., De Angelis, D., **Jakobson, K.**, Squeo, G., Caponio, F., Pasqualone, A., & Summo, C. (2026). Enhancing texturization of upcycled durum wheat meal protein through pH adjustment and low-moisture extrusion optimization. *Applied Food Research, 6*(1) 101925. doi.org/10.1016/j.afres.2026.101925

Author's contribution to the publications

I The author participated in the study design, characterization methodology development, data analysis, interpretation of results, and wrote the manuscript. The author further assembled and adapted these analytical methods into an integrated platform for assessing plant protein functionality at TFTAK.

II The author was involved in the study conceptualization, methodology development, experimental planning, conducting the experiments, data interpretation, and manuscript writing. Methodology development included an original scanning electron microscopy image-based visual assessment method for grading particle morphology.

III The author contributed to the interpretation of techno-functional analysis results and to the writing and revision of the manuscript.

IV The author was involved in data interpretation related to techno-functional, textural and sensory properties, and in writing, reviewing and editing of the manuscript.

Abbreviations

DA	Dairy alternatives
DWMP	Durum wheat meal protein
EA	Emulsification activity
ES	Emulsion stability
FA	Foam stability
FS	Foaming activity
HME	High-moisture extrusion
LAB	Lactic acid bacteria
LD	Laser diffraction
LME	Low-moisture extrusion
MA	Meat alternatives
OHC	Oil holding capacity
PS	Particle size
PSA	Particle size analysis
PSD	Particle size distribution
SEM	Scanning electron microscopy
TPA	Texture profile analysis
TVP	Texturized vegetable proteins
WHC	Water holding capacity
WSI	Water solubility index

Introduction

Global demand for dietary proteins is increasing, but animal-based dietary protein production is associated with relatively high greenhouse gas emissions and extensive land and water use. These high resource requirements, exceeding in fact planetary boundaries, challenge the scalability of animal-protein-rich diets for a projected global population of ten billion by 2050 (Fanelli, 2018; FAO, 2017; Valoppi et al., 2021; Von Koerber et al., 2017; Webb et al., 2023). Plant proteins have therefore emerged as an alternative protein source, offering substantially lower environmental impact alongside nutritional benefits such as dietary fibre and essential micronutrients (Sim et al., 2021). Consequently, their incorporation into food products has increased in recent years, particularly driven by the emergence of plant-based dairy alternatives (DA) and meat alternatives (MA), which account for a substantial share of recent product development activities (McClements & Grossmann, 2021).

Liquid plant-based DA are fundamentally colloidal systems in which plant-derived particles are dispersed within an aqueous continuous phase. The stability, structure, and sensory properties of these systems are determined by the physicochemical characteristics of the dispersed particles, including particle size (PS), morphology, solubility, and interfacial behaviour that determine technologically important emulsification, foaming, and textural properties. Therefore, understanding the functionality of plant protein ingredients is essential for selecting suitable materials and for achieving sensory and structural characteristics of the novel products suitable to replace the conventional dairy products (Aydar et al., 2020; Grossmann et al., 2021; McClements, 2020; Puleo et al., 2020).

Similarly, MA aim to reproduce the structure, texture, and juiciness of animal muscle products and commonly rely on texturized vegetable proteins (TVP) produced by extrusion to form fibrous, meat-like structures. Extrusion is the most widely applied technology for this purpose. The functional properties of the plant protein ingredients such as water- and oil-holding capacity and emulsifying ability are particularly important for MA applications, as they govern moisture and fat retention, texture formation, cooking yield, and determine how effectively plant proteins can support the structure and eating quality required for different meat alternative applications (McClements & Grossmann, 2022).

Despite their potential, broader use of plant proteins in the liquid and texturized food systems is constrained currently by their functional, sensory, and nutritional limitations (peculiarities). For instance, the limited solubility compromises other key functional properties required for stable structure formation (Zeng et al., 2022). Sensory challenges, including raw-material-specific off-flavours and a chalky, grainy, or gritty mouthfeel, limit consumer acceptance (Mittermeier-Kleßinger et al., 2021), and their amino acid profiles and digestibility are often inferior to those of animal proteins (Day et al., 2022; Ismail et al., 2020). These challenges are often exacerbated by industrial plant protein isolation processes, in which harsh conditions used, such as alkaline extraction and spray drying, cause protein denaturation and aggregation and may substantially reduce techno-functional performance, despite being essential from the point of industrial processing feasibility (Burger et al., 2022a; Dunford, 2012; Gutberlet, 2000; Karaca et al., 2011; Kyriakopoulou et al., 2021; Moreno et al., 2020; Taherian et al., 2011). While the properties of plant protein powders vary substantially with botanical origin, processing history, and purity, most existing research focuses on highly purified, laboratory-scale

protein isolates rather than industrially produced commercial ingredients (Burger & Zhang, 2019; Ma et al., 2022; McClements & Grossmann, 2021). However, industrially produced commercial powders constitute the primary raw materials used in plant-based food development. Therefore, systematic and application-relevant evaluations of these commercial ingredients are urgently needed to guide ingredient selection and formulation strategies in plant-based product development (Gastaldello et al., 2022).

This dissertation addresses the limited understanding of how the physicochemical characteristics of plant protein ingredients influence techno-functional performance and sensory perception relevant to dairy and meat alternative applications. Although plant proteins are widely used in such applications, there is limited knowledge of how variability in commercial protein powders translates into consumer-relevant product attributes. Moreover, the technological potential of modification strategies applied at the ingredient or product level remains inadequately understood in terms of their ability to purposefully tailor functional and sensory outcomes. To address these gaps, the present work adopts an approach combining comprehensive characterization of commercially available plant protein powders, targeted investigation of relationships between particle structure and sensory perception in liquid systems, and evaluation of fermentation and extrusion as technological modification strategies to better understand how raw material origin and technological processing conditions influence plant protein ingredient quality, with the aim of supporting the production of higher-quality plant protein ingredients that are more acceptable to consumers.

This dissertation is based on four publications. **Publication I** systematically characterized a broad range of commercial plant protein powders using multiple application-oriented methods and, for the first time, integrated inter-batch variability assessment into functional protein characterization. In **Publication II**, an original sensory-based visual grading approach for scanning electron microscopy images was introduced to evaluate particle morphology, establishing links between PS, surface features, and texture perception in liquid matrices. By using fermentation as a modification strategy, **Publication III** examined the integration of lactic acid fermentation into a pea protein isolation process, aiming to evaluate its effect on techno-functional properties and also on undesirable sensory characteristics, such as green, raw-material-specific notes, bitterness, and off-flavours. Finally, by applying low-moisture extrusion to restructure proteins into fibrous meat-like textures and as a modification strategy, **Publication IV** investigated both raw material selection and processing conditions, including the use of an upcycled durum wheat meal protein and pH adjustment, to study their influence on structure and texture development.

Overall, this dissertation advances the understanding of relationships between plant protein ingredient properties, processing strategies, and resulting techno-functional and sensory characteristics in application-relevant systems.

1 Literature review

1.1 Plant proteins

Proteins are essential macronutrients required for nutritional energy, maintaining muscle mass, supporting immune function, enabling cellular signalling, and facilitating tissue repair (Chandran et al., 2023). Plant-derived proteins have been recognized as sustainable and accessible sources of dietary protein next to and as alternatives to animal proteins. Their composition, structure, and physicochemical properties determine their nutritional quality and functional behaviour in foods. Compared to animal proteins, plant proteins generally provide a less balanced amino acid profile and exhibit lower digestibility, which is primarily attributed to their compact globular conformation and the presence of antinutritional compounds such as tannins and phytates (Day et al., 2022; Ismail et al., 2020). From a sensory perspective, plant protein isolates and concentrates may carry bitter, grassy, or beany off-flavours that originate from the raw material or develop during isolation and processing, often limiting the sensory quality of plant-based dairy alternatives (DA) and meat alternatives (MA) (Mittermeier-Kleßinger et al., 2021).

Structurally, most plant proteins are globular and classified according to solubility into albumins, globulins, prolamins, and glutelins. Most plant proteins are composed primarily of globulins and albumins, with globulins typically representing the dominant fraction. Albumins and globulins dominate in pulses and pseudocereals, whereas cereals are richer in prolamins and glutelins. In many legume proteins, globulins account for approximately 70% of total protein, while albumins contribute around 20%, with the remainder consisting of minor protein fractions such as convicilin, prolamins, and glutelins. Among globulins, legumin (11S) and vicilin (7S) are the most abundant storage proteins and are particularly important for techno-functional behaviour (Ma et al., 2022).

Functional behaviour is closely linked to compositional diversity. For example, albumins often show higher solubility at neutral to acidic pH due to their smaller molecular weight and greater flexibility, while globulins typically require modification to form gels. Globular proteins can act as effective surface-active agents in food systems, contributing to emulsification and foaming through partial unfolding and exposure of hydrophobic residues. Upon heating or other structural rearrangements, reactive groups may become exposed and form new intermolecular interactions, enabling protein aggregation or network formation that influences texture and stability. In contrast, prolamins and glutelins are capable of self-assembling into more organized networks upon hydration (McClements & Grossmann, 2021; Sim et al., 2021).

Plant protein ingredients are available as flours, concentrates, or isolates, which differ in protein concentration (roughly <50%, 50–80%, and >80%, respectively), molecular conformation, and aggregation state due to extraction and drying processes. Depending on processing history, proteins may remain native or become partially denatured and form aggregates, which typically reduces solubility while enhancing water-holding capacity (WHC) and viscosity. In addition, varying amounts of non-protein components such as starch, fibre, lipids, and minerals can further influence protein structure and interactions. Overall, the structure of plant proteins reflects a dynamic interplay between biological origin and processing conditions, and understanding protein composition, conformation, and aggregation behaviour is essential for predicting and optimizing functional performance in food applications (Ma et al., 2022).

A broad range of conventional plant sources can be used for protein extraction, including legumes such as soybean, pea, chickpea, lupin, and faba bean, cereals such as rice, wheat, millet, and barley, pseudocereals like amaranth, quinoa, and buckwheat, and various oilseeds, nuts, and other edible seeds (Sá et al., 2020). In addition to these more-established crops, unconventional vegetable proteins are increasingly obtained from leaves, stems, roots, algae, and grass crops, many of which originate from underutilized agricultural or processing side streams and therefore represent nutrient-dense and sustainable alternatives for food applications (Fang et al., 2024; Merlo et al., 2025; Procházka et al., 2023). Figure 1 provides a visual overview of commonly utilized plant sources for protein extraction, highlighting their broad agricultural and compositional diversity (Nikbakht Nasrabadi et al., 2021). Among the commonly used plant protein sources, soy remains the most dominant due to its established supply chains and well-documented functionality. However, allergenicity, phytoestrogen content, and sustainability concerns accelerated the search for alternatives (Fukushima, 2011; Medic et al., 2014; Patisaul & Jefferson, 2010). Pea proteins have emerged as the leading alternatives, with numerous commercial isolates available and expanding use in both MA and DA (Boukid, 2021a; Lu et al., 2020; Shevkani et al., 2019; Tulbek et al., 2017). Other crops such as fava bean, oat, canola, and potato are gaining attention, though their nutritional, sensory, and functional profiles remain less characterized (Boukid, 2021b, 2021a; Boukid & Castellari, 2022; Mäkinen et al., 2017; Spaen & Silva, 2021). The continued diversification and wider utilization of different plant protein sources not only broaden the ingredient base for plant-based foods but also contribute to agricultural sustainability through practices such as crop rotation (Mohler & Johnson, 2009).

The properties of plant proteins vary widely with botanical source, variety, and growing and processing history, and commercial ingredients can exhibit reduced functionality, compared to their lab-scale counterparts, due to the harsher, industrially applied processing conditions (Burger et al., 2022a; Karaca et al., 2011; Moreno et al., 2020). Systematic characterization of commercial plant protein ingredients is essential for understanding their variable functional and sensory performance. This knowledge supports their end-product-specific selection and the use of processing and modification strategies that are adjusted to the requirements of both the final product and the associated production process.

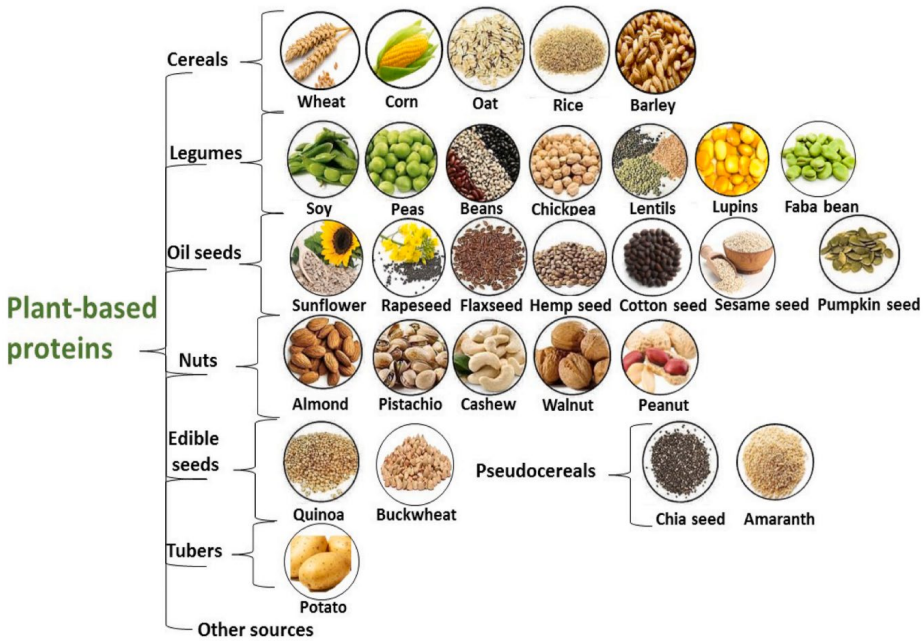

Figure 1. Main plant-based protein sources (Nikbakht Nasrabadi et al., 2021)

1.2 Meat- and dairy alternatives

The use of plant proteins in DA and MA is driven by their ability to replace the structural and techno-functional roles of animal proteins, making a clear understanding of their properties essential for the design of MA and DA. The functional requirements of plant proteins for DA and MA are determined by the nature of these fundamentally different food systems (De Angelis, Latrofa, Squeo, et al., 2024).

Plant-based DA aim to replicate the appearance, texture, sensory and stability attributes of conventional dairy products. Liquid plant-based DA include a range of different products, such as beverages, yogurts, creams, coffee creamers, fermented drinks, custards, and desserts. Fundamentally, liquid DA products are colloidal systems in which plant-derived particles are dispersed within an aqueous continuous phase. The properties of these systems are governed by the physicochemical properties of the dispersed particles including size, morphology, solubility, emulsification, foaming, and rheological structuring. These properties govern dispersion stability, resistance to phase separation, viscosity and structure development, and mouthfeel in beverages, yogurts, and emulsified products. A clear understanding of these functional attributes is therefore essential for selecting appropriate plant proteins and for achieving dairy-like sensory and structural quality (Aydar et al., 2020; Grossmann et al., 2021; McClements, 2020; Puleo et al., 2020). Insufficient solubility or interfacial activity can compromise system stability, while inappropriate gelation or viscosity development may negatively affect sensory quality (De Angelis, Latrofa, Squeo, et al., 2024; Ma et al., 2022). The production of plant-based DA, particularly milk alternatives, can follow either a traditional whole-seed approach or a formulation-based approach using plant protein ingredients. The traditional method involves milling of soaked or dry seeds, aqueous extraction, removal of insoluble material, homogenization, and heat treatment, but the composition of the

final product is largely dictated by the raw material and often results in beverages with relatively low protein content when starch-rich pulses are used. To overcome these limitations, a formulation-based strategy using plant protein concentrates or isolates has been developed, in which a protein dispersion is prepared and combined with oil to form a pre-emulsion, followed by high-pressure homogenization to reduce droplet size and improve colloidal stability, and subsequent heat treatment to ensure microbial safety and shelf-life stability (Vogelsang-O'Dwyer et al., 2021).

MA, in contrast, aim to mimic the structure, texture, and juiciness of animal muscle tissue, requiring plant proteins to form cohesive, elastic, and aligned structures. For these applications, plant proteins are used both as primary structuring materials in the form of texturized vegetable proteins (TVP) and as binders or extenders in minced, hybrid and emulsified systems. As most plant proteins are inherently globular and lack intrinsic fibrousness, their functionality for TVP production must be optimised through ingredient selection and processing. Among available structuring technologies, extrusion is the most established and widely applied approach, using controlled heat, pressure, and shear to transform globular plant proteins into fibrous networks. For MA, WHC and oil-holding capacity (OHC), gelling and emulsifying properties are particularly important, as they govern moisture and fat retention, texture formation, cooking yield, and juiciness. These properties collectively determine the ability of plant proteins to form cohesive, fibrous matrices during processes such as extrusion or to function effectively as binders and extenders in comminuted systems. Application-specific requirements for different techno-functional properties in DA and MA are illustrated in Figure 2.

Figure 2. Key techno-functional properties for different DA and MA (De Angelis, Latrofa, Caponio, et al., 2024).

Despite the widely recognized importance of techno-functional properties, relatively few studies have systematically examined specific or threshold values of individual functional attributes as explicit success criteria for defined food applications. Instead, most studies address these properties indirectly, inferring their relevance from processing behaviour or final product performance, or by generally stating that high levels of certain functionalities are required. Nevertheless, the available literature clearly demonstrates

that the functional properties of plant protein powders play a decisive role in shaping the structural, textural, and sensory characteristics of both DA and MA. The following section summarizes how key functional properties of plant protein raw materials, particularly WHC, viscosity development, and structuring behaviour, influence the performance and quality attributes of dairy and meat alternative end products.

In dairy alternative systems, the role of plant protein functional properties is commonly discussed through their contribution to viscosity development, gel formation, and water retention. Montemurro et al. (2021) reviewed plant-based yogurt alternatives and highlighted that product texture and stability are largely governed by fermentation-driven gelation, protein–polysaccharide interactions, and WHC; however, the intrinsic techno-functional properties of the protein ingredients were not explicitly quantified (Montemurro et al., 2021). Similarly, Lorusso et al. (2018) investigated yogurt-like products based on fermented quinoa flour and attributed viscosity development and WHC primarily to starch gelatinization and fermentation-induced structural changes (Lorusso et al., 2018). Taken together, these studies indicate that in fermented DA, functional outcomes such as gel strength, viscosity, and stability are central to process and product development, even though they are rarely traced back directly to the functional properties of the raw materials.

In contrast, clearer relationships between raw material functional properties and end-product performance have been demonstrated for MA produced by extrusion. Kaleda (2021) showed that powders with higher intrinsic WHC (pea-rich blends, ≈2.35 g/g) consistently yielded extrudates with higher WHC (up to ≈2.6 g/g), indicating that the water-binding capacity of the raw material is largely retained after extrusion despite protein denaturation and aggregation. Sensory moistness was also related to WHC, although even stronger associations were observed with OHC and extrusion-induced porosity. This demonstrates that powder functionality defines the upper limit of water retention, while processing-driven structural features ultimately govern perceived juiciness. These findings provide quantitative evidence that intrinsic functional properties of protein powders translate directly into functional attributes of meat alternative products (Kaleda et al., 2021).

At a broader level, Kyriakopoulou et al. (2021) emphasized the central importance of WHC and OHC, gelling, emulsification, and texturization behaviour for achieving meat-like structure and sensory quality in plant-based MA. However, their review primarily discussed these properties at a conceptual level and in relation to formulation and processing strategies, without providing systematic quantitative links between intrinsic protein powder functionality and specific product outcomes (Kyriakopoulou et al., 2021).

Collectively, the available literature demonstrates that although the functional properties of plant protein raw materials are widely recognized as critical for both DA and MA, their effects are most often inferred indirectly, with only a limited number of studies providing explicit, quantitative links between raw material functionality and end-product performance. Consequently, there is a clear need to investigate the functional properties of plant protein powders under application-relevant processing conditions, as adopted in the approach of the present work.

1.3 Techno-functional properties

1.3.1 Solubility and water solubility index

Protein solubility and dispersibility are among the most decisive functional properties governing the performance of plant proteins in liquid and semi-solid food systems. Solubility describes the fraction of the material that is molecularly dissolved in water, whereas dispersibility refers to the ability of the entire powder to distribute uniformly in an aqueous medium without forming visible aggregates or sediment (Ebert et al., 2020). These properties are particularly critical for applications in which plant proteins must be present as stable colloidal or molecularly dispersed phases, such as plant-based beverages, milk alternatives, drinkable yogurts, soups, and emulsified systems, where undissolved particles lead to sedimentation, phase separation, and undesirable gritty mouthfeel. In this context, protein solubility is also tightly linked to other techno-functional attributes, including emulsification, foaming, and gelation, because only solubilized proteins can effectively adsorb to interfaces, unfold, and form viscoelastic interfacial films (Zeng et al., 2022; Zhao et al., 2020).

At the molecular level, protein solubility is governed by the balance between attractive protein–protein interactions and stabilizing protein–water interactions. Proteins in aqueous systems are thermodynamically driven to minimize free energy by burying hydrophobic amino acid residues within their interior while exposing hydrophilic and charged residues toward the surrounding solvent. This spatial organization allows hydration shells to form around the protein, which stabilizes the dispersed state. However, hydrophobic residues that remain exposed on the surface, create patches that promote intermolecular association, thereby reducing solubility.

Electrostatic forces also play a central role: at pH values above or below the isoelectric point (pI), proteins carry a net negative or positive charge, respectively, generating electrostatic repulsion between molecules that favours solubilization. In contrast, at the pI the net charge approaches zero, minimizing repulsive forces and allowing attractive interactions such as hydrophobic interactions, hydrogen bonding, and van der Waals forces to dominate. This shift promotes protein–protein association, leading to aggregation and precipitation. Once aggregates exceed a critical size, they sediment, which macroscopically manifests as reduced solubility (Lam et al., 2018). While many plant proteins exhibit pI values in the acidic range, approximately pH 4 to 5, cereal storage proteins display markedly higher pI values. Wheat gluten proteins, for example, have pI values of approximately pH 8.1 for gliadin, pH 7.1 for glutenin, and pH 7.5 for total gluten, whereas zein, the principal storage protein in maize, has a pI near pH 6.2. These elevated pI values contribute to solubility behaviours that differ substantially from those of legume or other seed storage proteins. The high pI of cereal prolamins is primarily attributed to their amino acid composition, which is characterized by high proline and glutamine content, low charge density, and a reduced proportion of acidic residues (Curioni et al., 1990).

Several environmental and compositional factors modulate this molecular balance. pH is the primary determinant, but ionic strength, temperature, and solvent composition also exert strong effects. Dissolved salts compress the electric double layer surrounding proteins by screening surface charges, which lowers the zeta-potential and reduces electrostatic repulsion. Certain ions, such as sulphate, hydrogen phosphate, ammonium, and potassium, further disrupt hydration layers by strengthening ion–water interactions, thereby exposing hydrophobic regions and promoting aggregation. Temperature

influences solubility in a dual manner: moderate heating (up to approximately 50 °C) can increase molecular mobility and hydration, but higher temperatures destabilize non-covalent bonds within the protein, causing partial unfolding and exposure of hydrophobic groups, which accelerates aggregation and precipitation. These effects are particularly relevant in industrial processing, where thermal treatments and pH shifts are frequently applied (Lam et al., 2018).

The intrinsic surface properties of plant proteins strongly influence their solubility behaviour. Proteins with higher surface hydrophobicity and lower surface charge generally exhibit reduced solubility, as is the case for pea protein isolates compared with soy, chickpea, faba bean, or lentil proteins (Lam et al., 2018). During wet extraction, exposure to extreme pH values and elevated temperatures can induce denaturation, which unfolds the native globular structure and exposes previously buried hydrophobic moieties, thereby increasing surface hydrophobicity and decreasing solubility (De Angelis, Latrofa, Caponio, et al., 2024; De Angelis, Latrofa, Squeo, et al., 2024). By contrast, milder fractionation approaches such as dry fractionation tend to better preserve native protein conformations, although they produce ingredients with lower protein purity (Assatory et al., 2019). Processing history therefore largely determines whether a protein remains in a soluble, functional state or becomes aggregated and poorly dispersible.

It is essential to distinguish between protein solubility and overall powder solubility. Overall solubility refers to the fraction of the entire material that dissolves in water, whereas protein solubility expresses the proportion of protein that is solubilized relative to the total protein present (Hopf et al., 2024). Plant protein concentrates and flours often contain substantial amounts of non-protein components, including starch, soluble carbohydrates, minerals, and fibres, which can contribute to the water solubility index (WSI) and thus elevate apparent solubility values without reflecting true protein dissolution (De Angelis, Latrofa, Caponio, et al., 2024; De Angelis, Latrofa, Squeo, et al., 2024). Conversely, insoluble dietary fibres can reduce WSI and impair dispersibility even when part of the protein fraction is soluble. WSI therefore integrates the contributions of all hydrophilic components remaining in the aqueous phase after hydration or heating, whereas protein solubility specifically captures the behaviour of the protein fraction. This distinction is highly relevant for applications such as beverages, where non-protein solubles may improve dispersion but do not substitute for truly solubilized proteins in delivering emulsification and mouthfeel.

Commercial plant protein powders generally exhibit much lower protein solubility than animal-derived proteins, which limits their functionality in liquid foods (Zeng et al., 2022). Many commercial isolates and concentrates display solubility values below 20% at neutral pH, although potato and rapeseed proteins engineered for beverage applications represent notable exceptions (Ebert et al., 2020). Mechanical and physicochemical treatments can partially overcome these limitations. For example, high-pressure homogenization has been shown to increase pea protein solubility from approximately 10% to over 50% by reducing surface hydrophobicity and breaking up aggregates, although solubility remains low near the pI (Burger et al., 2022a). Strong correlations between solubility, emulsifying capacity, and water absorption further demonstrate that solubility is a central determinant of techno-functional performance in plant protein systems (Zhao et al., 2020).

Taken together, solubility emerges as a prerequisite for the successful use of plant proteins in applications where proteins must be molecularly dispersed, such as plant-

based milks, beverages, and fermented DA. The inherently pH-dependent and processing-sensitive nature of plant protein solubility, combined with the confounding influence of non-protein components in powders, explains why achieving stable, smooth, and homogeneous products remains challenging. Optimizing extraction, fractionation, and post-processing strategies to preserve favourable surface properties and minimize aggregation is therefore essential for improving the functional performance of plant protein ingredients in aqueous food systems.

1.3.2 Water- and oil-holding capacity

WHC and OHC are fundamental functional properties describing the ability of proteins to retain water and oil, respectively, within their three-dimensional matrix when exposed to external forces such as gravity, centrifugation, or processing stresses. WHC is commonly defined as the amount of water absorbed or retained per gram of protein and is frequently referred to in the literature as hydration, water-binding, or water-absorption capacity, whereas OHC represents the amount of oil that can be immobilized per gram of protein material. These properties are critically important in food systems because water often accounts for more than half of the total product mass and lipids are major carriers of flavour and mouthfeel. Therefore, inadequate WHC leads to water loss and undesirable texture, while insufficient OHC compromises fat stabilization and sensory quality. At the molecular level, WHC arises from the capacity of protein molecules to associate with water through a combination of ion–dipole, dipole–dipole, dipole-induced dipole, and hydrophilic interactions, whereby water molecules bind to charged amino acid side chains, peptide backbone groups, amide moieties, hydroxyl groups, and even nonpolar residues, each contributing differently to overall hydration. Proteins with a high density of charged and polar groups show enhanced electrostatic attraction toward water, and WHC is strongly modulated by amino-acid composition, molecular conformation, and the architecture of the protein matrix, particularly pore size and molecular weight distribution (Kaushik et al., 2016; Oikonomou & Krokida, 2012). A critical feature governing WHC is pH, because hydration reaches a minimum at the isoelectric point where net charge is zero and protein–protein interactions dominate over protein–water interactions, promoting aggregation and water expulsion. For example, for mineral acid casein curd, WHC was lowest at pH ≈ 5.3, with a value of approximately 2.4 g water/g dry casein. When the pH was shifted away from the isoelectric point, WHC increased markedly, reaching about 5.2 g water/g dry casein at pH 4.3. This increase in WHC at lower or higher pH values is attributed to enhanced electrostatic repulsion between protein molecules, which promotes network expansion and greater water retention (Teo et al., 1996). Soy protein gels prepared at pH 2.75 exhibited a WHC of approximately 90–95%, whereas gels prepared closer to the pI at pH 3.50 showed a lower WHC of about 70–80% under identical conditions. The higher WHC at pH 2.75 is attributed to increased electrostatic repulsion between protein molecules, leading to a more homogeneous and compact gel network capable of retaining more water (Puppo & Añón, 1998). Conversely, WHC increases at low salt concentrations due to the ability of ions to attract and organize water around protein molecules, and it is further enhanced by denaturation processes that unfold proteins and expose previously buried hydrophilic groups. This mechanism explains why thermally treated proteins and isolates obtained by alkaline extraction and isoelectric precipitation exhibit higher WHC than those recovered by ultrafiltration, as processing severity increases molecular flexibility and surface polarity (Stone et al., 2015). In practice, these effects translate into

significant functional differences between ingredients: soy protein isolates consistently show higher WHC than pea protein isolates, while chickpea, fava bean, and oat proteins tend to exhibit lower values, reflecting both inherent botanical differences and processing-induced structural changes (Fuhrmeister & Meuser, 2003). Such variations are highly relevant in product design, particularly for meat alternatives, where high WHC has been directly linked to improved juiciness and a more desirable fibrous texture in extruded products (Kaleda et al., 2021).

In contrast, OHC is governed primarily by hydrophobic interactions between the aliphatic chains of lipids and the nonpolar side chains of amino acids, meaning that proteins with greater surface hydrophobicity display a higher affinity for oil binding (Li et al., 2021). The ability of a protein to entrap and stabilize oil is not determined solely by its chemical composition but also by the physical characteristics of the protein–lipid matrix, including porosity, interfacial area, and the spatial distribution of oil droplets. Droplet size and stability, as well as the presence of emulsifying agents, further modulate OHC by influencing how effectively lipids are dispersed and immobilized within the protein network (Lam et al., 2018; Stone et al., 2015). These molecular and structural features explain the wide variability in OHC reported for plant protein isolates, which depends on crop type, cultivar, and processing history. Among commercial ingredients, canola and potato proteins are frequently reported to exhibit the highest OHC, while oat proteins generally show the lowest, again highlighting the combined influence of raw material composition and technological treatment (Fuhrmeister & Meuser, 2003). Because OHC is closely linked to emulsifying capacity, it plays a particularly important role in applications where proteins function as fat binders, such as in meat alternatives, where oil retention directly affects mouthfeel, lubrication, and flavour release, or in other fat-rich foods where stable emulsions are required.

Taken together, WHC and OHC represent complementary aspects of protein functionality that determine how water and lipids are structured within food matrices and thus govern both processing performance and final product quality. Their dependence on amino-acid composition, molecular conformation, hydrophilic–hydrophobic balance, and matrix architecture explains why even proteins derived from the same crop can behave differently when produced by different technologies (Kaushik et al., 2016; Oikonomou & Krokida, 2012).

WSI (as explained in Section 1.3.1), WHC, and OHC can be measured not only for plant protein powders but also for milled TVP, while WHC and WSI can additionally be determined for whole TVP. Assessing these functional properties at different processing stages is particularly relevant, as similar trends in WHC, WSI, and OHC have been observed in raw materials and the resulting TVP. Furthermore, from MA applications perspective, this is especially important for WHC, since the WHC of plant protein powders plays a crucial role in sensory attributes, being closely associated with higher perceived moisture, juiciness, tenderness, texture, and overall mouthfeel in meat alternative products (Latrofa et al., 2025). This suggests that analysing the properties of raw materials prior to extrusion can provide valuable insights into the characteristics of the final extruded products. In particular, WHC and WSI measurements in whole extrudates are used to investigate the physical entrapment of water and to simulate their behaviour during rehydration and subsequent application in food formulations (Lee et al., 2022)). Additionally, knowledge of WHC is essential for predicting the amount of water required during the extrusion process, thereby supporting process optimization and product consistency (De Angelis, Latrofa, Caponio, et al., 2024).

1.3.3 Emulsification and foaming

Emulsification refers to the ability of surface-active molecules, such as proteins, to facilitate the formation and stabilization of a system in which one immiscible liquid (most commonly oil) is dispersed as fine droplets within another (usually water). In food systems, emulsification is a critical functional property because it underpins the physical stability, texture, and sensory quality of products such as plant-based DA, sauces, dressings, desserts, and composite foods. The meaning of emulsification in this context extends beyond simple droplet formation: it encompasses the capacity of proteins to adsorb at the oil–water interface, reduce interfacial tension, and generate a protective interfacial layer that limits droplet aggregation, flocculation, and coalescence over time (Friberg, S., Larsson, K., & Sjoblom, 2003; Mustakova et al., 2025).

At the molecular level, emulsification is governed by the amphiphilic nature of proteins, which contain both hydrophilic and hydrophobic amino acid residues. Upon homogenization, proteins migrate to the newly created oil–water interface and orient themselves so that hydrophobic regions interact with the oil phase while hydrophilic regions remain in the aqueous phase. This adsorption minimizes interfacial free energy and leads to the formation of a viscoelastic interfacial film. Structurally, adsorbed proteins adopt so-called train, loop, and tail configurations: train segments lie flat along the interface, whereas loops and tails extend into the continuous phase, contributing steric hindrance and electrostatic repulsion between droplets. The strength, thickness, and cohesiveness of this interfacial film are central determinants of emulsion stability (ES), as they define the system's resistance to mechanical stress and droplet coalescence (Friberg, S., Larsson, K., & Sjoblom, 2003; Lam et al., 2018).

Several intrinsic protein properties control emulsifying performance. Protein solubility is fundamental, as only soluble protein fractions can rapidly diffuse to and saturate the interface, thereby ensuring uniform droplet coverage and the formation of small droplet sizes. Molecular flexibility is considered one of the most critical characteristics of an effective emulsifier: flexible proteins can unfold and rearrange more readily at the interface, leading to stronger interfacial films. In contrast, rigid globular proteins adsorb more slowly and require greater conformational adjustment, which can limit their emulsifying efficiency. Surface hydrophobicity and amino acid composition further modulate adsorption behaviour, with polar residues favouring interfacial stabilization in emulsions and hydrophobic residues enhancing affinity for the oil phase (De Angelis, Latrofa, Caponio, et al., 2024; Stone et al., 2015).

Environmental conditions strongly influence emulsification through their effects on protein charge and intermolecular interactions. Emulsions are generally more stable at pH values away from the protein isoelectric point, where proteins carry a net charge that generates electrostatic repulsion between droplets. Under these conditions, droplets remain farther apart, reducing attractive forces between proteins adsorbed on neighbouring droplets and thereby decreasing the likelihood of flocculation. At the same time, repulsion between droplets can promote stronger lateral interactions among proteins adsorbed to the same droplet, reinforcing the integrity of the interfacial film. Conversely, near the isoelectric point or at high ionic strength, electrostatic repulsion is diminished, droplets approach more closely, and attractive forces dominate, making the emulsion more prone to instability and coalescence (Lam et al., 2018; Peng et al., 2016). Processing-induced structural changes also play a key role in emulsification. Moderate heat treatment can enhance emulsifying properties by partially unfolding proteins, exposing hydrophobic groups, and increasing interfacial film thickness. However,

excessive denaturation may promote protein–protein aggregation in the bulk phase, resulting in uneven interfacial coverage and the formation of inhomogeneous films that weaken ES and increase susceptibility to flocculation (Benjamin et al., 2014). Particle size (PS) is another important factor: finer protein particles exhibit improved accessibility to the oil–water interface and faster adsorption kinetics, which generally translates into improved emulsification compared with coarser fractions (De Angelis, Latrofa, Caponio, et al., 2024).

Beyond proteins themselves, non-protein components naturally present in plant-derived ingredients can significantly influence emulsification. Dietary fibres and other polysaccharides may enhance ES indirectly by increasing the viscosity of the continuous phase, thereby slowing droplet movement and reducing collision frequency. Such components can act as secondary stabilizers or thickeners, complementing the interfacial role of proteins and contributing to the overall physical robustness of the emulsion system(De Angelis, Latrofa, Caponio, et al., 2024).

From an application perspective, emulsification is essential for the functionality of plant proteins as binders, stabilizers, and texture modifiers in complex food matrices. The ability to tailor emulsifying properties through protein source selection, PS control, pH adjustment, ionic strength management, and thermal processing enables the design of stable, appealing, and clean-label products.

1.3.4 Particle size

PS and particle size distribution (PSD) are fundamental descriptors of plant protein powders, influencing dissolution, dispersion, mixing and extrusion behaviour, powder flowability, stability, and end-product quality (Barbosa-Cánovas, 2005). Larger or polydisperse particles sediment more rapidly and can destabilize suspensions, while smaller or more uniform particles contribute to improved homogeneity. Industrial-scale protein powders often display reduced solubility due to denaturation and aggregation during harsh processing, which can result in the formation of larger undissolved particles compared to lab-prepared isolates (Burger et al., 2022b; Karaca et al., 2011; Moreno et al., 2020; Taherian et al., 2011). In protein concentrates, additional carbohydrates and fibres can remain undissolved, further increasing the particulate load in liquid systems (Jia et al., 2021). Application conditions also affect PS: during heat treatment, homogenization, and acidification near the isoelectric point, proteins aggregate more readily, increasing the risk of particle formation in fermented DA (Klost & Drusch, 2019; Ma et al., 2022; Pua et al., 2022).

Laser diffraction (LD) is one of the most widely used techniques for particle size analysis (PSA). The principle is based on the inverse relationship between PS and scattering angle when particles pass through a laser beam. A typical LD system includes a laser source, detectors for scattered light, and a dispersion unit that delivers a homogenous and reproducible sample flow. PSD is derived by fitting scattering data to optical models, typically reporting parameters such as D10, D50, and D90, along with mean diameters D[4,3] and D[3,2] (Bancarz et al., 2007). LD is non-destructive and suitable for both dry powders and dispersions, provided that dispersant, concentration, and refractive index parameters are properly set. The method has been applied to compare dry- versus wet-fractionated ingredients and to assess the effects of spray drying, which can induce unfolding, aggregation, and the formation of larger, irregular particles (Alonso-Miravalles et al., 2019; Haque & Adhikari, 2015; Vicente et al., 2013; Vogelsang-O'Dwyer et al., 2020).

Instrumental PSA therefore provides essential information for predicting functional properties and guiding formulation of plant-based foods. However, size data alone cannot fully explain sensory perception, making it necessary to combine PSA with morphological and mechanical assessments to understand particle-related texture in liquid products.

1.3.5 Particle morphology

Plant protein particle morphology, including shape, surface roughness, porosity, and degree of agglomeration, strongly influences dispersion, solubility, processing, and the sensory quality of liquid and semi-solid products (McClements & Grossmann, 2021; Sim et al., 2021). Low solubility and aggregation can yield undissolved particulates that are perceived as grit ty, a key negative attribute in plant-based DA (Grossmann et al., 2021; McClements, 2020; McClements et al., 2019; Paul et al., 2020). Industrial harsher processing compared with lab-scale often increases denaturation/aggregation, while the presence of non-protein solids in concentrates means powder solubility can diverge from protein solubility (Burger et al., 2022a; Ebert et al., 2020; Jia et al., 2021; Karaca et al., 2011; Moreno et al., 2020; Taherian et al., 2011). Heat treatment, homogenization, and acidic pH typical for fermentation further promote aggregation near the isoelectric point (Klost & Drusch, 2019; Ma et al., 2022; Pua et al., 2022).

Scanning electron microscopy (SEM) provides high-contrast images of plant protein powders, revealing how extraction and drying shape particle surfaces and integrity: wet-fractionated, spray-dried isolates commonly appear smooth and rounded, whereas dry-fractionated concentrates are more irregular and angular; processing of cereal flours can likewise reduce agglomeration and yield more spherical particles (Alonso-Miravalles et al., 2019; He et al., 2020; Kumar et al., 2022; Vogelsang-O'Dwyer et al., 2021). However, dry-state SEM cannot indicate which particles persist in aqueous dispersions, so morphology must be linked to dispersion behaviour.

Crucially, perceived grittiness depends on particle hardness, edge sharpness, concentration, and matrix viscosity, not size alone; harder, angular particles are detectable at smaller sizes, and lower-viscosity matrices heighten roughness sensations (Appelqvist et al., 2015; Engelen et al., 2005; Imai et al., 1995; Petersson et al., 2013; Shewan et al., 2020; Tyle, 1993).

1.4 Sensory properties of plant protein powders

Sensory properties are decisive for the acceptance of plant-based foods, as texture, taste, and aroma collectively shape consumer preference. Plant-based dairy and meat alternatives are commonly evaluated against their animal-derived counterparts, where smoothness, creaminess, juiciness, and a relatively neutral flavour profile serve as the benchmark. However, sensory limitations are widely recognised as the main bottleneck for the broader adoption of plant proteins in these applications. Plant protein ingredients frequently exhibit texture, odour and taste defects that significantly reduce consumer acceptance (Grossmann et al., 2021; Jaeger et al., 2024; McClements, 2020; McClements et al., 2019; Moss et al., 2022; Paul et al., 2020; Vaikma et al., 2025). Figure 3 illustrates that plant proteins, based on trained sensory analysis, are often described as beany, bitter, astringent, earthy, green, musty, soapy, acrid, metallic, or nutty. These attributes frequently co-occur, giving rise to a complex and predominantly negative flavour and mouthfeel profile that contrasts strongly with the smoother and more neutral sensory characteristics of animal-based products (Sarkar, 2024).

The present section focuses on two aspects that are particularly critical for plant-based food systems: first, the role of PS, hardness, and aggregation state in driving gritty or chalky perception in liquid applications; and second, the contribution of taste- and aroma-active compounds to bitterness, beany/grassy notes, and astringency in plant protein powders.

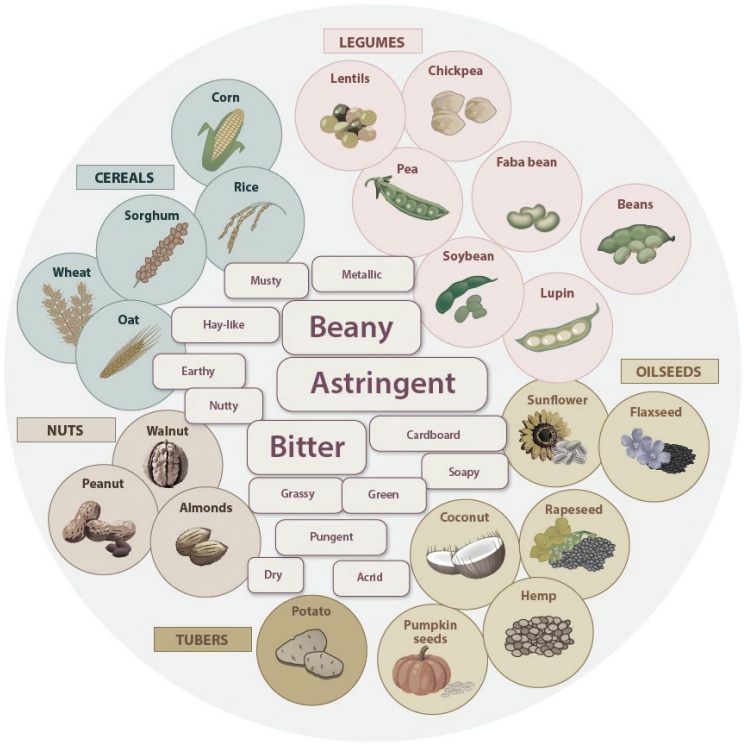

Figure 3. Undesirable sensory attributes of commonly studied plant protein sources (Sarkar, 2024).

1.4.1 Sensorially-perceived particle size

The perception of undissolved particles is one of the key factors influencing the sensory quality of plant-based DA. In literature, this attribute is often described using terms such as grainy, chalky, sandy, or gritty; for consistency, the term gritty is adopted here (Grossmann et al., 2021; McClements, 2020; McClements et al., 2019; Paul et al., 2020). Products with a gritty texture are typically rated lower in consumer preference studies, whereas smooth and creamy mouthfeel is associated with higher acceptance (Jaeger et al., 2024; Moss et al., 2022; Vaikma et al., 2025).

Research shows that PA is a major determinant of gritty perception, but it does not act alone. Detection thresholds increase with PS and concentration, yet the surrounding matrix can modify perception. For example, grittiness is more pronounced in low-viscosity matrices where particles are freely dispersed, while thicker or more viscous matrices can mask the sensation (Imai et al., 1995; Petersson et al., 2013). In model systems, carrot particles were undetectable at mean sizes of 56–92 µm but became clearly perceived as grainy at 225 µm (Appelqvist et al., 2015). Similarly, studies with hard, angular garnet particles showed detection thresholds as low as 11–22 µm, while soft, round polyethylene particles of up to 80 µm were not perceived as gritty (Tyle,

1993). These examples illustrate that hardness, shape, and solubility also play roles in detection, but PS remains the primary predictor of perception.

In plant protein powders, poor solubility and aggregation frequently lead to the presence of undissolved particles in dispersions, especially under acidic pH conditions where solubility is lowest (Ma et al., 2022; McClements & Grossmann, 2021). Such particles not only sediment but also contribute directly to gritty mouthfeel. Importantly, concentrates that contain carbohydrates and fibres may add to the particulate load beyond proteins alone (Jia et al., 2021). Thus, controlling PS and PSD, through both raw material selection and processing strategies such as homogenization or fermentation, is essential for improving the sensory quality of plant-based DA.

1.4.2 Taste and aroma

Taste and aroma are among the most significant sensory challenges in the use of plant proteins for DA and MA. Unlike animal proteins, which are generally perceived as neutral in flavour, plant proteins frequently carry undesirable off-notes that reduce consumer acceptance. These include bitterness, astringency, chalkiness, and raw-material-specific beany or grassy notes, all of which are regularly reported as barriers to consumer liking (Day et al., 2022; Ismail et al., 2020; Mittermeier-Kleßinger et al., 2021; Sim et al., 2021).

Off-flavours in plant proteins originate from both non-volatile and volatile compounds formed or released during raw material processing and protein isolation. Non-volatile contributors such as saponins, alkaloids, and phenolic compounds are commonly associated with bitterness and astringency, while hydrophobic peptides generated during protein hydrolysis or harsh isolation conditions can further intensify bitter taste perception (Ismail et al., 2020; Roland et al., 2017; Schindler et al., 2012). In addition, carbohydrates and fibres present in protein concentrates may contribute to chalky aftertastes and mouth-drying sensations (Jia et al., 2021).

Volatile compounds play a dominant role in shaping plant protein aroma and typically include aldehydes, ketones, alcohols, esters, and pyrazines. Among these, aldehydes, particularly those derived from lipid oxidation and lipoxygenase activity, are strongly associated with green, grassy, and beany odours. Processing and storage conditions can further intensify off-flavour perception by releasing, concentrating, or transforming flavour-active compounds, thereby promoting the formation or accumulation of volatile compounds and making off-flavours especially pronounced in commercial plant protein isolates (Roland et al., 2017; Schindler et al., 2012).

Within this broader context of plant protein sensory challenges, pea protein stands out as one of the most widely used ingredients in plant-based foods. Yet, pea-based products are frequently described as beany, grassy, and earthy, often accompanied by bitterness and astringency (Mittermeier-Kleßinger et al., 2021). These sensory attributes reflect the combined impact of non-volatile bitterness-contributing compounds and volatile aroma compounds described above, with aldehydes playing a particularly prominent role. Hexanal, in particular, is widely recognised as a key marker compound underlying pea-like odour, while additional alcohols, ketones, esters, and pyrazines contribute green, earthy, or roasted notes (Roland et al., 2017; Schindler et al., 2012).

To address these challenges, strategies such as fermentation and enzymatic treatment have been increasingly applied to pea protein, with studies demonstrating reduced bitterness and lower aldehyde concentrations following microbial modification (Mittermeier-Kleßinger et al., 2021). However, despite these advances, pea protein

remains challenging in terms of taste and aroma, indicating that existing approaches are not yet sufficient to achieve consistently acceptable flavour quality.

1.5 Plant protein modification technologies

The limited solubility, textural defects, and off-flavours of plant proteins remain major barriers to their broader application in dairy and meat alternatives. To overcome these challenges, various modification strategies have been explored, aiming to improve functionality, structure, and sensory quality. Among these, extrusion and fermentation stand out as the most widely studied and applied approaches. Extrusion is primarily used to restructure proteins into fibrous textures resembling meat, also changing sensory and techno-functional properties, while fermentation improves nutritional, functional, and sensory properties through microbial activity. The following subsections provide an overview of these two technologies.

1.5.1 Fermentation

Fermentation has long been used to improve the nutritional quality, safety, and shelf-life of plant-based foods, and its application to protein ingredients has gained new momentum with the rise of plant-based dairy and meat alternatives (Akharume et al., 2021). Depending on the technique, fermentation is carried out either as solid-state fermentation, with limited water, or as submerged fermentation, with high water content. Solid-state fermentation is often preferred in industry due to its higher yield, lower costs, and favourable flavour development.

Microorganisms commonly used include lactic acid bacteria (LAB), *Bacillus* spp., yeasts, and filamentous fungi. These act through several mechanisms: they degrade antinutritional compounds (saponins, tannins, phytates, enzyme inhibitors), release bioactive compounds (e.g., isoflavones, vitamins), and modify protein functionality by partial hydrolysis. Fermentation has been shown to improve solubility, emulsification, and foaming capacity (FC) in some plant proteins, while reducing bitterness and undesirable “beany” notes typical of legumes (Mittermeier-Kleßinger et al., 2021). It has also been applied to improve texture in vegan cheese alternatives, increase the creaminess of plant-based yogurts, and enhance flavour in beverages alternatives (Akharume et al., 2021).

1.5.2 Extrusion

Extrusion is one of the most widely applied technologies for structuring plant proteins, enabling the conversion of globular proteins into fibrous meat alternatives (McClements & Grossmann, 2021; Sim et al., 2021). Figure 4 illustrates a schematic representation of a co-rotating twin-screw extruder commonly used for this purpose (Schmid et al., 2022). In this thermomechanical process, proteins are subjected to heat, mixing, pressure, and shear within a screw-driven barrel, before being shaped through a die. The combination of thermal denaturation and mechanical alignment unfolds protein molecules, disrupts hydrogen and disulfide bonds, and promotes the formation of new inter- and intramolecular interactions (hydrogen, disulfide, hydrophobic), resulting in aggregated networks and fibrous textures (McClements et al., 2021). Alignment of unfolded proteins during shearing is followed by solidification in a die, which locks in the fibrous anisotropic structure characteristic of meat alternatives.

Extrusion also improves the nutritional and safety profile of plant proteins by reducing antinutritional factors and increasing amino acid availability (Sim et al., 2021). Process

outcomes are determined largely by the specific mechanical energy (SME) input, which depends on extruder configuration (screw, die), process parameters (temperature, moisture, screw speed, pressure), and material composition (water content, pH, protein/starch ratio) (Beck et al., 2017; Sman & Goot, 2023; Vatansever et al., 2024). For example, moisture acts as a plasticizer that controls viscosity, expansion, and fibrousness of the extrudates (De Angelis, Latrofa, Caponio, et al., 2024). pH and ionic strength also influence texturization by modulating disulfide bond formation and protein–protein interactions (Cheftel et al., 1992; Muhialdin & Ubbink, 2023; Nisov et al., 2022).

Both low-moisture extrusion (LME) and high-moisture extrusion (HME) are used to produce TVP. LME (moisture < 40%) produces expanded, porous structures stabilized by evaporation, while HME (moisture > 40%) relies on long cooling dies and laminar flow to create dense, fibrous textures closer to whole muscle meat (McClements et al., 2021; Schmid et al., 2022). LME offers simplicity, high throughput, and cost efficiency, whereas HME provides superior fibrousness and mouthfeel, though with higher equipment and energy costs. Both are central technologies in commercial meat alternative production.

Overall, extrusion and fermentation represent two complementary strategies: extrusion provides structural transformation and fibrousness, while fermentation modulates flavour, nutrition, and functionality. Their combined use offers great potential for overcoming current limitations in plant proteins and achieving consumer-acceptable DA and MA.

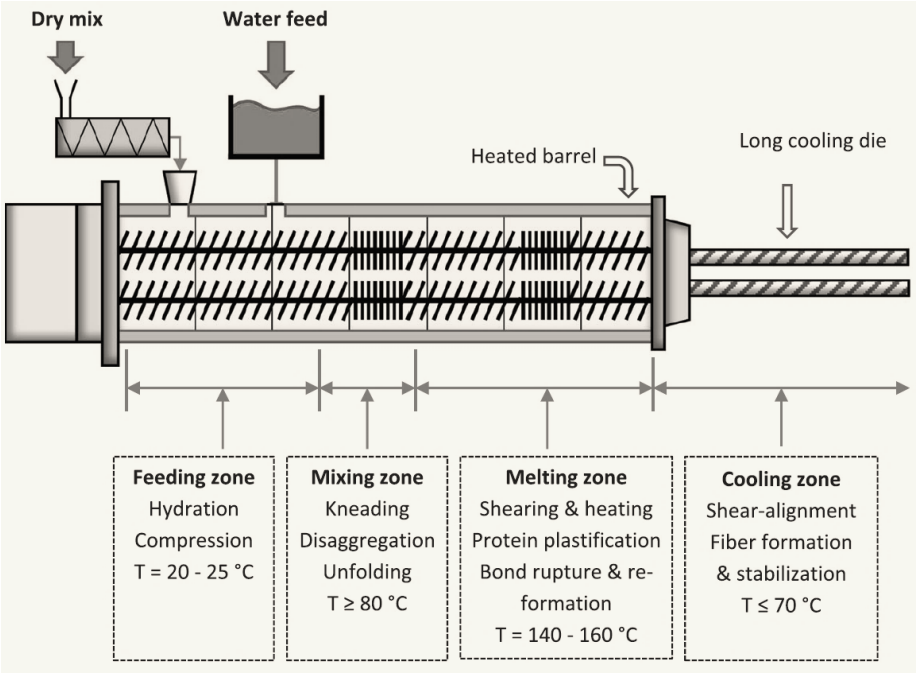

Figure 4. A schematic representation of a co-rotating twin-screw extruder (Schmid et al., 2022).

1.6 Texture properties of TVP

1.6.1 Sensorially perceived texture of the extrudates

The sensorially perceived texture of extrudates is a decisive quality attribute for consumer acceptance of plant-based meat alternatives. Extrusion enables the transformation of globular plant proteins into fibrous structures, which are designed to mimic the anisotropic texture of animal muscle (McClements & Grossmann, 2021; Sim et al., 2021). From a sensory perspective, the degree of fibrousness, juiciness, cohesiveness, and chewiness determines whether extrudates are perceived as realistic and palatable. Products that fail to replicate the structural cues of meat are often described as dry, crumbly, or pasty, which negatively affects consumer liking (McClements et al., 2021).

Several functional properties of proteins directly influence the sensory perception of extrudates. WHC plays a central role in determining juiciness: proteins with higher WHC help retain moisture within the fibrous matrix, preventing dryness and improving succulence (Kaleda et al., 2021). OHC and emulsification capacity further contribute to lubrication and mouthfeel by stabilizing fat within the protein network. Poor solubility and aggregation, by contrast, can hinder protein alignment during extrusion, resulting in coarse or grainy textures. Processing conditions, including temperature, shear, and moisture content, strongly modulate these interactions. For instance, LME yields porous, expanded structures with a spongy bite, while HME produces denser, layered fibrous textures closer to muscle meat (McClements et al., 2021).

The perception of extrudate texture is thus the outcome of both intrinsic protein characteristics and process parameters. While functional properties such as solubility, WHC, and OHC establish the foundation for texturization, extrusion design and conditions govern how these properties are translated into sensory qualities such as fibrousness, juiciness, and chewiness. Consequently, optimizing both raw material selection and processing strategy is essential to achieve consumer-acceptable textures in plant-based meat alternatives.

1.6.2 Instrumentally measured texture profile of the TVP

In addition to hydration and interfacial properties, the mechanical behaviour of plant proteins can be assessed through instrumental texture analysis, which provides objective measures complementing sensory evaluation. Among these methods, Texture Profile Analysis (TPA) is the most widely used for characterizing both conventional foods and texturized plant-based products (Schreuders, Schlangen, Kyriakopoulou, Boom, & Jan van der Goot, 2021). The method simulates mastication by subjecting the sample to two consecutive compressions, generating a force-time curve from which primary parameters, hardness, cohesiveness, springiness, adhesiveness, and resilience, and secondary parameters, gumminess or chewiness, are calculated (Khule et al., 2024). These metrics correspond to mechanical responses that relate to sensory perception: hardness indicates the force needed to deform the sample, cohesiveness reflects internal structural integrity, and springiness describes the ability to recover after compression (Hong et al., 2022).

Initially developed for gels and meats, TPA has become a standard tool for assessing TVP, providing reproducible data that help link extrusion conditions and formulation to perceived texture (Schreuders, Schlangen, Kyriakopoulou, Boom, & van der Goot, 2021). However, the heterogeneous and anisotropic nature of extruded products can affect repeatability, underscoring the importance of standardized test conditions such as

sample geometry, compression rate, and rehydration protocol (Khule et al., 2024; Peleg, 2019).

Overall, TPA offers a practical and quantitative means to evaluate the mechanical texture of plant protein extrudates and, when combined with sensory and physicochemical analyses, supports the optimization of processing parameters for desirable product quality.

2 The aims of this dissertation

Literature review identified multiple peculiarities and issues associated with plant protein application in meat and dairy alternative products, and differences in techno-functional and sensory performance compared to animal proteins. It can be hypothesized that these challenges can be addressed by selecting ingredients with more appropriate properties targeted towards specific products. Therefore, the overall aim of this dissertation was to characterize commercially available plant protein ingredients, their physicochemical, techno-functional, and sensory properties, and to test two technological modification strategies to address the key challenges limiting their application in alternative meat and dairy products. The specific aims of the four studies (publications) could be characterized as follows:

1. To comprehensively characterize the techno-functional and sensory properties of a wide range of commercially available plant protein powders in the context of their suitability for meat and dairy alternative products.
2. To investigate how particle size and surface morphology of plant protein powders determine the sensory perception of graininess in water dispersions, with a focus on their implications for liquid food applications.
3. To explore lactic acid fermentation during pea protein isolation as a method to reduce undesirable raw-material-specific flavours and off-notes and to investigate the effect on the techno-functional properties of the resulting plant protein ingredient.
4. To investigate how variation in extrusion parameters can be used to modify the physicochemical, techno-functional, and sensory properties of texturized plant-based meat alternatives.

3 Materials and methods

The work involved both commercially available protein powders (**Publication I, II, IV**) as well as lab-scale produced isolates (**Publication III**), assessed using a range of physicochemical, techno-functional, texture, and sensory properties, and processing methods. The studies addressed both ingredient-level properties (evaluating the properties of the powders) and product-level performance in the context of alternative meat (producing and evaluating TVP) and dairy (evaluating the powders in liquid matrix) applications. The methods applied across the studies are presented below in structured sections, referencing the respective publication where each methodology was used. Detailed information on the materials and specific methodologies used in each publication can be found in the individual publications (Appendices 1–4).

3.1 Plant protein powders

Across the publications, plant protein powders from a diverse range of botanical sources were utilized, including pea, oat, fava bean, mung bean, chickpea, potato, canola, soy, and wheat. These were mainly in the form of isolates (>80% protein) and concentrates (56–60% protein) (**Publication I-IV**) with one exception in **Publication IV** where dry-fractionated defatted durum wheat meal protein (DWMP) enriched flour was additionally employed.

In **Publication I**, 24 commercial plant protein isolates and concentrates (17 isolates, 7 concentrates) were used for techno-functional and sensory characterization. The appearance and the nutritional composition of these products are presented in Figure 5 and Table 1.

*Figure 5. A photo of commercial protein powders used in the publication (**Publication I**).*

*Table 1. Nutritional composition according to manufacturers' specifications and native pH. Samples marked with asterisks are different production batches of the same product (**Publication I**).*

Sample	Protein, % dwb	Fat, % dwb	Carbohydrates, % dwb	Ash, % dwb	Native pH
Canola	90	-	-	5	6.13
Chickpea 1	89	0	1	4	6.95
Chickpea 2	89	0	2	3	6.95
Fava bean 1	60	4	25	-	6.54
Fava bean 2	60	4	-	-	6.70
Fava bean 3	88	5	1	6	6.45
Fava bean 4	60	3	18	-	6.72
Mung bean	85	4	1	3	7.07
Oat 1	59	9	27	4	6.01
Oat 2*	56	13	21	3	6.55
Oat 3*	56	13	21	3	6.55
Oat 4*	56	13	21	3	6.61
Pea 1	85	-	-	5	7.35
Pea 2	85	9	0	6	7.39
Pea 3	84	9	0	5	7.46
Pea 4	80	5	2	9	7.10
Pea 5	90	-	-	4	7.56
Pea 6*	80	9	4	5	7.02
Pea 7*	80	9	4	5	6.60
Pea 8*	80	9	4	5	7.10
Pea 9*	80	9	4	5	7.11
Potato	90	0	0	5	7.32
Soy	90	1	-	6	7.74
Wheat	82	5	8	1	6.26

In **Publication II**, a subset of powders from **Publication I** was analysed for PS and morphology characteristics, both sensorially and instrumentally.

In **Publication III**, the protein isolates were prepared in the lab from Estonian-grown pea flour variety "Kirke" via alkaline solubilization and chemical and fermentation-based precipitation.

In **Publication IV**, a 75:25 blend of commercial pea isolate with protein content 80% (Caremoli S.p.A, Monza, Italy) and defatted DWMP with protein content 28.53% (provided by Casillo Next Gen Food srl, Corato, Italy) was used for the extrusion experiments.

3.2 Fermentation-assisted pea protein powder production

In **Publication III**, fermentation was employed for two distinct purposes: (1) as an innovative isoelectric precipitation step during plant protein isolation from raw material

(pea flour), enabling in-house production of pea protein isolates for techno-functional characterization; and (2) to investigate the effects of lactic acid fermentation on the sensory, structural, and techno-functional properties of pea protein isolates. This approach enabled evaluation of fermentation as a processing strategy for improving the application potential of plant protein ingredients.

3.2.1 Starter cultures

Five commercial LAB starter cultures (SC1–SC5) with distinct bacterial compositions were initially screened for suitability in pea protein fermentation, based on manufacturer recommendations. The cultures comprised strains of *Streptococcus*, *Lactobacillus*, *Bifidobacterium*, *Lactiplantibacillus*, and *Pediococcus*.

Preliminary small-scale fermentations were conducted in pea protein solutions to assess acidification performance and overall fermentation behaviour. Based on these screening experiments, two starter cultures were selected for further studies: SC1, which exhibited the fastest acidification, and SC2, which provided superior sensory properties.

3.2.2 Protein solubilization

Pea protein extraction was adapted from previously established methods with minor optimization based on preliminary trials. Pea flour was mixed with distilled water (1:6 ratio) at 21 °C and adjusted to pH 7.5 using 2 M NaOH. The suspension was stirred for 1 hour, with pH maintained through periodic NaOH additions. Following centrifugation to remove insoluble material, the supernatant was divided to produce three isolates: one control using chemical precipitation (PPI_Ctrl) and two via fermentation (PPI_SC1 and PPI_SC2).

3.2.3 Protein precipitation using LAB fermentation

SC1 and SC2 were used to inoculate the pea protein solution preheated to 40 °C in 5 L bottles. After adding 1% starter stock, fermentation was carried out at 40 °C under stirring until the pH reached 4.8. The suspension was then centrifuged to recover the protein paste, which was resuspended in distilled water (1:1), neutralized to pH 7.0 with 2 M NaOH, and lyophilized.

3.2.4 Chemical protein precipitation

Isoelectric precipitation was performed by adjusting the pH of the pea protein solution to 4.8 using 4 M HCl. The precipitated protein was recovered by centrifugation, resuspended in distilled water (1:1), neutralized to pH 7.0 with 2 M NaOH, and lyophilized. The resulting protein powder served as the control.

3.3 Extrusion trials

In **Publication IV** the extrusion trials were performed to produce TVP with tailored structural and functional properties relevant to meat alternative applications. By modifying key processing parameters and protein blend composition during LME, the publication aimed to elucidate how extrusion conditions influence texture formation, fibrousness, and material functionality of the resulting TVP. The produced TVP served as model systems for assessing structure–function–sensory relationships relevant to plant-based meat applications.

3.3.1 Preparation of the blends and experimental design

One protein blend consisting of pea protein isolate and DWMP (75:25; 65.7% protein in the blend) was used in extrusion trials. A 2^3 reduced factorial design was applied, with randomized experimental order and one replicate (S7_1 and S7_2) to strengthen statistical reliability. The experimental plan is presented in Table 2. The independent variables were moisture content (28% and 32%), screw speed (400 and 600 rpm), and pH (6.9 and 7.5), with pH adjusted by in-line addition of 1.5% KOH solution. Two reference samples, 100% pea protein isolate (PPI) and an 80:20 mix of pea protein isolate and pea starch concentrate (PPI_PSC, 66.3% protein), were also extruded under pre-established laboratory conditions for comparison.

*Table 2. The experimental design for extrusion of texturized vegetable proteins in **Publication IV**.*

Trial	Total moisture (%)	Screw speed (rpm)	pH
S1	28	400	6.9
S2	28	600	6.9
S3	32	400	6.9
S4	32	600	6.9
S5	28	400	7.5
S6	28	600	7.5
S7_1	32	400	7.5
S7_2	32	400	7.5
S8	32	600	7.5

3.3.2 Extrusion process

Extrusion was performed using a co-rotating intermeshing twin-screw extruder (KETSE 20/40, Brabender GmbH, Germany) with a temperature profile of 40/76/122/135/152/154 °C, optimized based on preliminary trials. Powder mass flow was calibrated prior to extrusion, and water along with 1.5% KOH solution was added through separate inlets using calibrated peristaltic pumps. Extrusion was conducted through a 1.6 mm die, and samples were collected once process conditions (temperature, pressure, torque) stabilized. Products were dried at 70 °C for 50 minutes until water activity dropped below 0.6, then sealed in zip-lock bags for storage.

3.4 Techno-functional and texture properties of the powders and TVP

3.4.1 Preparation of the heat-treated plant protein powder water dispersions without and with acidification step

To simulate industrial conditions, heat treatment and acidification were applied prior to selected analyses in **Publication I** and **II** to evaluate the functional properties of the protein powders under technologically relevant processing conditions.

Heat-treated water dispersions of the plant protein powders (without and with acidification step) were prepared for the heat-treatment-based solubility analysis (**Publication I**) and for instrumental and sensory PS analysis and SEM analysis

(**Publication II**). Six percent (w/v) powder dispersions were heat-treated at 85 °C for 15 minutes and subsequently cooled to room temperature in a water bath. From their native pH the samples were acidified to pH 4.5 with 10% lactic acid to prepare the acidified samples.

3.4.2 Solubility of plant protein powders in heat-treated water dispersions

Water solubility of plant protein powders in heat-treated water dispersions was determined in **Publication I** gravimetrically at native and acidified pH (4.5) using filtered commercial drinking water to simulate industrial conditions. Following centrifugation and washing, the precipitates were dried, and solubility was calculated as the proportion of dried pellet mass relative to the initial powder on a dry weight basis. Therefore, our heat-treatment-based solubility method evaluates the solubility of the whole powder after pasteurization at both native pH and pH 4.5, thereby capturing solubility changes induced by thermal processing and acidification. In contrast, the WSI determines the fraction of the powder that dissolves in water at room temperature and native pH and thus reflects the intrinsic dispersibility of the powder under mild, cold-water conditions. Accordingly, WSI provides information on solubility under non-processed conditions, whereas water solubility of plant protein powders in heat-treated water dispersions characterizes functional solubility under processing conditions relevant to industrial food applications.

3.4.3 Water solubility index, water holding capacity, oil holding capacity

One gram of powder was mixed with 10 mL of either distilled water or rapeseed oil, followed by gentle mixing, centrifugation, and decanting. The weight of the retained precipitate was used to calculate water- and oil-holding capacities (WHC and OHC), expressed as grams of liquid retained per gram of powder (dwb). The supernatant from the water-based samples was oven-dried to determine the WSI, calculated as the percentage of dissolved solids relative to the initial powder weight (dwb). WHC, OHC, and WSI can be measured for both: powders and TVP. In Publication I and III, the WHC, OHC, and WSI were measured in the powders. In Publication IV, WHC and WSI were determined for raw materials prior to extrusion and for both whole and milled TVP after extrusion, whereas OHC was measured for raw materials and milled extrudates only, as large TVP particles cannot be reliably analysed for OHC. For milling, extrudates were ground using a coffee mill to obtain a uniform PS.

3.4.4 Foaming and emulsifying properties

Foaming properties (**Publication I and III**) were evaluated by dispersing protein powder in water and homogenizing to incorporate air. FC was calculated based on the volume increase after frothing, and foam stability (FS) was assessed by measuring the remaining foam volume after 1 h standing period.

For emulsifying properties (**Publication I and III**), protein powder was dispersed in a water and oil mixture and homogenized to form an emulsion. Emulsification activity (EA) was determined by the proportion of the emulsified layer after centrifugation. ES was assessed following heat treatment (in a water bath at 80 °C for 30 min), cooling (in an ice-water bath for 15 min), and a second centrifugation, based on the height of the emulsified layer.

3.4.5 Particle size distribution

PSD (**Publication II**) was measured using two LD methods. Dry powders were analysed with a PSA 1190 (Anton Paar, Austria) using a dry jet dispersion unit under standard settings. Heat-treated (85 °C for 15 minutes) six percent (w/v) plant protein powder water dispersions
at native pH and pH 4.5 were assessed with a Mastersizer 3000 (Malvern Instruments, UK) equipped with a wet dispersion unit. Ultrapure water was used as the dispersant, either unmodified or acidified to pH 4.5 with lactic acid. Samples were measured under gentle stirring (500 rpm) to prevent particle breakdown and maintain comparability with sensory evaluation. Results are reported as volume-weighted D10, D50, and D90 values, representing the particle diameters below which 10%, 50%, and 90% of the total particle volume fall, respectively.

3.4.6 Scanning electron microscopy

In **Publication II**, particle morphology was imaged using an environmental scanning electron microscope (EVO LS15, Zeiss, Germany). Heat-treated (85 °C for 15 minutes) six percent (w/v) plant protein powder water dispersions at native and acidified pH were frozen in liquid nitrogen, lyophilized, and mounted as a monolayer on carbon tape-coated holders. Samples were sputter-coated with a 1.5 nm layer of gold/palladium (80:20) and imaged at 15 kV using secondary electron detection at 70 Pa, with magnifications of ×200 and ×500.

3.4.7 Texture-profile analysis of the TVP

TPA was performed in **Publication IV** on rehydrated extruded TVP samples using a texture analyser equipped with a flat compression plate and load cell. Whole extrudates were rehydrated in warm water, blotted dry, and placed in a uniform layer on the testing platform. Samples were compressed twice under controlled speed and compression settings to evaluate mechanical properties. Parameters including hardness, resilience, cohesiveness, springiness, and chewiness were calculated.

3.5 Sensory analysis

Across **Publications I–IV**, a range of sensory evaluation approaches were applied to characterize the sensory and structural properties of plant protein powders and TVP. In **Publications I and III**, trained panellists evaluated odour, taste, and mouthfeel-based particle texture attributes of protein powder dispersions, including overall intensity, raw-material-specific odour and taste, off-notes, bitterness, astringency, perceived PS, and particle quantity. **Publication II** focused on mouthfeel-based PS perception of the heat-treated (85 °C for 15 minutes) six percent (w/v) plant protein powder water dispersions at different pH conditions and additionally introduced a visual sensory assessment of particle morphology based on SEM images, covering surface roughness, angularity, and heterogeneity. In **Publication IV**, sensory evaluation was applied to rehydrated TVP, assessing odour and taste intensity alongside a comprehensive set of texture attributes, including fibrousness, springiness, hardness, chewiness, adhesiveness, moistness, and granularity. Together, these sensory tests allowed comparison of sensory quality and perceived structural properties across different plant protein powders and their processing conditions.

All sensory analyses were conducted at TFTAK (Tallinn, Estonia) in a sensory evaluation facility compliant with ISO 8589:2007. The panel consisted of trained assessors with prior experience in evaluating plant-based protein products, in accordance with ISO 8586:2012 and 8586:2023. All participants provided informed consent, were briefed on the purpose and procedures of the research and were in good health with no known allergies to the tested materials.

3.5.1 Odour, taste, and mouthfeel-based particle texture of plant protein powders

In **Publication I**, the samples were prepared without the heat treatment and only at their native pH as 6% (w/v) dispersions in potable water and served at room temperature. For evaluation, each dispersion was poured into sniffing glasses and covered with lids to minimize volatile compound loss. Samples were vigorously mixed prior to pouring to ensure homogeneity. A designated plant protein sample was included as a reference standard in all sensory sessions.

In **Publication I**, sensory evaluation of the powders covered three modalities: odour, taste, and texture. Odour and taste assessments included overall intensity, raw-material-specific odour and taste (e.g., cereal- or legume-like), off-notes, bitterness, and astringency. Texture properties of the plant powders in liquid matrix in Publication I were evaluated based on perceived particle size and the amount of particles. Panellists could also provide optional open-ended comments.

Attributes were rated using a 10-point scale (0–9). For odour and taste, the scale ranged from 0 = "none" to 9 = "very strong." For texture, PS was rated from 0 = "particles missing" to 9 = "very big," and particle quantity from 0 = "particles missing" to 9 = "mostly particles." Data were collected using RedJade sensory software (RedJade Sensory Solutions LLC, Martinez, CA, USA).

In Publication II, the mouthfeel-based evaluation of PS was conducted on the heat-treated water dispersions of the powders at native and acidified pH (4.5), assessed at room temperature. The remaining mouthfeel-based sensory procedure followed the same methodology as described in **Publication I**, with the exception that only PS was evaluated; odour and taste attributes were excluded.

In **Publication III**, the same sensory evaluation protocol was applied as in **Publication I**, with the only difference that attributes "raw-material" odour and taste were replaced with "pea" odour and taste to target the legume-specific sensory characteristics of the samples.

3.5.2 Odour, taste, and mouthfeel-based texture properties of the TVP

In **Publication IV**, sensory properties of the TVP were assessed, not the powders. TVP were rehydrated in tap water at 60 °C for 60 minutes, and excess water was blotted dry before evaluation. Sensory assessments were conducted at 21 °C in clear plastic cups. A structured 10-point scale (0 = "none", 5 = "moderate", 9 = "very strong") was used. The evaluation included odour, taste, and texture modalities. Odour and taste were assessed for overall and raw material intensity. Texture attributes included fibrousness (assessed tactilely by hand), and springiness, hardness, chewiness, adhesiveness, moistness, and granularity (assessed orally).

3.5.3 Quantitative visual assessment of SEM images

In **Publication II**, two distinct sensory tests were conducted: (I) a mouthfeel-based PS evaluation (described above in Section 3.5.1), and (II) a visual assessment of particle morphology based on SEM images. Accordingly, Publication II introduces this novel visual grading approach for assessing particle morphology using SEM images. The SEM image assessment panel consisted of different assessors trained in visual analysis. The assessment covered three visual attributes: surface roughness, angularity, and heterogeneity. Roughness described the degree of surface unevenness of individual particles, angularity reflected the irregularity of particle shape, and heterogeneity indicated the variation in PS and shape within a sample. For each attribute, standardized reference images representing scale anchors were provided. Attributes were rated on a 10-point scale (0–9), where 0 corresponded to completely smooth, circular, or homogeneous particles and 9 to completely rough, angular, or heterogeneous particles, respectively. Two SEM images per sample were evaluated for each attribute to account for sample variability. Higher magnification images (×500) were used for assessing roughness and angularity, while lower magnification images (×200) were used to support heterogeneity evaluation. Panellists based their ratings on the overall appearance of visible particles, excluding fibre-like fragments. All assessments were performed in two replicated sessions. The methodological development and evaluation process of our novel SEM-based visual grading approach to particle morphology are discussed in detail in Section 4.2.1.

3.6 Statistical analysis

In Publication I, solubility, oil- and water-holding capacities were analysed in triplicate, while emulsification and foaming measurements were conducted in duplicate. Mean values and standard deviations were calculated. Data analysis and visualization were performed using R version 4.3.0. LOESS regression was applied to gelling and visco-thermal data. Spearman's rank correlations were computed using the R "correlation" package (v0.8.4) and visualized with R "corrplot" (v0.92).

In Publication II, data were analysed and visualized using R version 4.3.0. Results are reported as means with standard deviations. LOESS regression (span = 1.1) was used to model D90 as a function of sensory PS, and breakpoint analysis was performed using the R "segmented" package (v2.0-1). Multiple linear regression assessed the relationship between sensory perception, particle morphology, and size distribution, with model quality evaluated using the R "performance" package (v0.10.8). Spearman correlations were calculated using the R "correlation" package (v0.8.4).

In Publication III, starter culture screening was performed in biological duplicates. Protein content, WHC, OHC, WSI, foaming, and emulsification properties were measured in analytical triplicates, while sensory profiles were analysed in duplicates. Data analysis and visualization were conducted using R version 4.3.0. Kruskal–Wallis tests followed by Conover–Iman post hoc comparisons with Bonferroni adjustment were applied using the "conover.test" package (v1.1.5).

In Publication IV, all data are presented as means ± standard deviation. Statistical significance was evaluated using one-way ANOVA followed by Tukey's HSD test in Minitab 19. Differences between experimental samples and both raw materials and reference products were assessed using Dunnett's multiple comparisons test. WHC was measured in triplicate; TPA was performed in eight replicates; and sensory analysis was conducted in two sessions. Response surface models were generated using Chemometric

Agile Tool (version 05.09.2023). Model fit and coefficient significance were evaluated by residual analysis, and visualizations were created in R version 4.3.0.

In all the statistical tests, the significance level α was set to 0.05.

4 Results and discussion

This section provides an overview of the core results obtained in **Publications I–IV**. These publications address complementary challenges related to the functionality and sensory performance of plant protein ingredients, beginning with the analysis of market-level variability of the commercial plant protein powders and extending to targeted modifications at the ingredient and processing levels. Detailed descriptions of these findings are available in the full texts of the respective publications, included as Appendices 1–4. In the final subsection, the scientific and practical impact of the work is critically evaluated, followed by a discussion of the main limitations and perspectives for future research.

4.1 Techno-functional and sensory characterization of commercial plant protein powders

Publication I aimed to comprehensively characterize the techno-functional and sensory properties of a wide range of commercially available plant protein powders, in order to provide an understanding of the current market situation and evaluate their suitability for applications in DA and MA. This publication addresses a critical knowledge gap and generates insights that can support the targeted use of plant protein powders in specific food applications.

This publication focused on commercially available powders from emerging crops, with soy and wheat included for comparison. To capture raw material and processing variation, different production batches of the same pea and oat protein products were also analysed (Ma et al., 2022).

4.1.1 Solubility of plant protein powders in heat-treated water dispersions and water solubility index

In industrial food applications, plant protein powders are frequently exposed to thermal processing and, in many cases, to acidification, such as during pasteurization and fermentation steps in dairy-type products. These processing steps can substantially affect protein solubility, aggregation behaviour, and particle characteristics, effects that are not adequately reflected by solubility measurements conducted under mild, cold-water conditions. Consequently, in this study, powder solubility was evaluated using three methods: WSI at room temperature and native pH, and two heat-treatment methods assessing solubility at both native and acidic pH 4.5. While WSI is commonly reported in research, the heat-treatment methods better reflect industrial practice, where products are pasteurized and many DA are formulated near pH 4.5. It is important to distinguish between whole powder solubility, which is the approach applied in the present thesis, and protein solubility, which is more commonly reported in plant protein research and literature. However, when considering the applicability of plant protein powders in end-use applications, it is essential to evaluate the solubility of the entire powder rather than only the protein fraction. While highly purified isolates consist predominantly of protein and most functional properties originate from this fraction, plant protein concentrates also contain carbohydrates that possess their own functional properties. For example, these components may contribute to sediment formation in liquid matrices.

The results are presented in Figure 6, where dots show WSI values and lines indicate solubility after heat treatment. Overall, solubility at room temperature was low (average 28%), with canola and potato isolates being exceptions, showing nearly complete solubility. Wheat, oat, most pea samples, and one fava bean isolate exhibited the lowest solubility (<10%), whereas other fava bean concentrates reached 40–60%. Batch-to-batch variation was especially high in pea proteins, with up to a fourfold difference, while oat powders were more consistent.

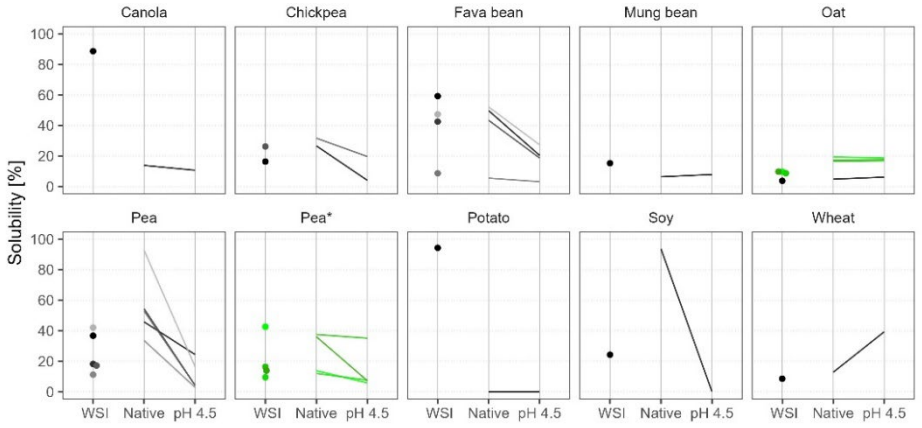

*Figure 6. Water solubility of commercial plant protein powders evaluated using three different methods. The water solubility index (WSI) was determined at room temperature at the native pH of the dispersions. The other methods included a heat treatment step and two pH conditions: native pH and pH 4.5. Individual dots and lines correspond to different products, green colour and asterisks denote different batches of the same product (**Publication I**).*

Heat treatment generally improved solubility at native pH, most notably in pea proteins, where values increased by up to 36 percentage points, with some samples (Pea 5 and soy) reaching 93% solubility. In contrast, oat, wheat, and chickpea improved only modestly, while canola and potato formed heat-induced gels. At acidic pH 4.5, solubility declined sharply, often by threefold, particularly in pea and soy, while poorly soluble proteins such as oat and mung bean were less affected. Wheat solubility increased at acidic pH, reflecting its isoelectric point near neutral pH.

Large batch-to-batch differences, particularly in pea proteins, pose a challenge for industry, as product performance may not be reproducible across production batches. These findings are consistent with earlier reports of poor solubility in commercial plant protein powders (Burger et al., 2022a; Ebert et al., 2020). Together, the results highlight solubility as a major limitation of plant protein ingredients, requiring additional processing steps to achieve functionality in liquid applications.

4.1.2 Water and oil holding capacity

Water holding capacity (WHC) and oil holding capacity (OHC) reflect the ability of proteins to retain water or oil, properties influenced by protein size, composition, conformation, and the presence of carbohydrates or fats (Kaushik et al., 2016; Li et al., 2021). Proteins rich in hydrophilic groups typically exhibit higher WHC through hydrogen bonding, while hydrophobic side chains facilitate oil interactions.

As shown in Figure 7, soy displayed by far the highest WHC (6.3 g H_2O g^{-1}), followed by Pea 5 (3.5 g H_2O g^{-1}), both of which also had high solubility at neutral pH. In contrast, most pea samples varied widely (0.9–3.5 g H_2O g^{-1}), while chickpea, fava bean, mung bean, oat, and wheat generally showed values below 2.6 g H_2O g^{-1}, with oat and wheat having the lowest (~1.5 g H_2O g^{-1}). Canola and potato WHC could not be measured, as they dissolved almost completely.

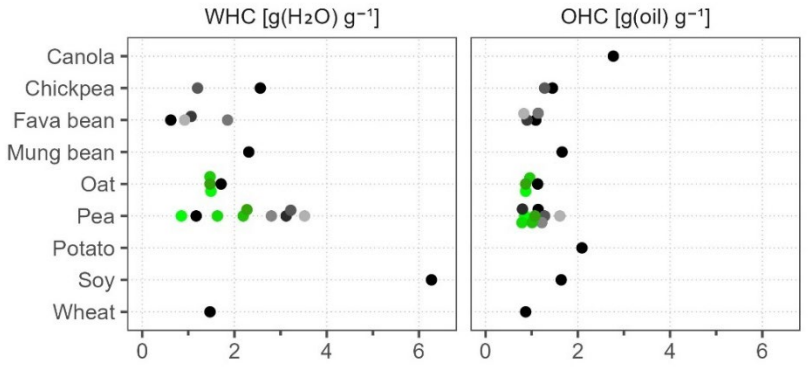

*Figure 7. Water- and oil holding capacity (WHC and OHC) of commercial plant protein powders. Each dot represents an individual product, green symbols indicate different batches of the same product (**Publication I**).*

OHC values were lower and less variable across samples, ranging between 0.8–1.7 g oil g^{-1}, except for canola (2.8 g oil g^{-1}) and potato (2.1 g oil g^{-1}), which exhibited the highest capacities. Batch-to-batch variation was minor for both pea and oat proteins.

These findings are consistent with earlier reports that soy exhibits superior WHC compared to other plant proteins (Fuhrmeister & Meuser, 2003; Zhao et al., 2020). Importantly, WHC strongly affects the juiciness of plant-based MA, where values above 3 g H_2O g^{-1} are considered desirable (Kaleda et al., 2021). In this publication, only soy and three pea samples met this threshold, underscoring the challenge of replacing soy in applications requiring high water retention.

4.1.3 Emulsification and foaming properties

Foaming and emulsification are key functionalities for plant-based products such as dressings, sauces, ice cream, and dairy or egg alternatives, as they depend on protein adsorption at air–water or oil–water interfaces, which stabilizes bubbles or droplets through repulsive forces and interfacial rigidity (McClements & Grossmann, 2021).

As shown in Figure 8, FC varied widely among samples. Wheat (98%) and potato (95%) displayed the highest FC, followed by chickpea and canola, whereas oat had the lowest values (9–19%). Interestingly, solubility did not fully explain these differences: despite equally poor solubility, wheat showed excellent FC while oat did not, suggesting additional factors such as molecular flexibility and hydrophobicity play an important role (Zayas, 1997). In contrast, FS showed a different pattern: mung bean produced the most stable foam despite low FC (25%), aligning with earlier findings (X. Tang et al., 2021). Potato and wheat, though strong in FC, generated unstable foams, while oat again performed poorly. Pea samples showed moderate FC and variable FS (31–68%), with modest batch-to-batch differences.

EA was generally around 50% across samples, except for oat and mung bean (<23%), confirming their poor emulsification. Increasing protein concentration did not improve EA, consistent with prior reports that industrial isolates perform worse than lab-scale ones functionality (Jiang et al., 2015; X. Tang et al., 2021). ES, which included a heat-treatment step, improved slightly in some samples, most notably in canola (68%), but remained low for oat and mung bean.

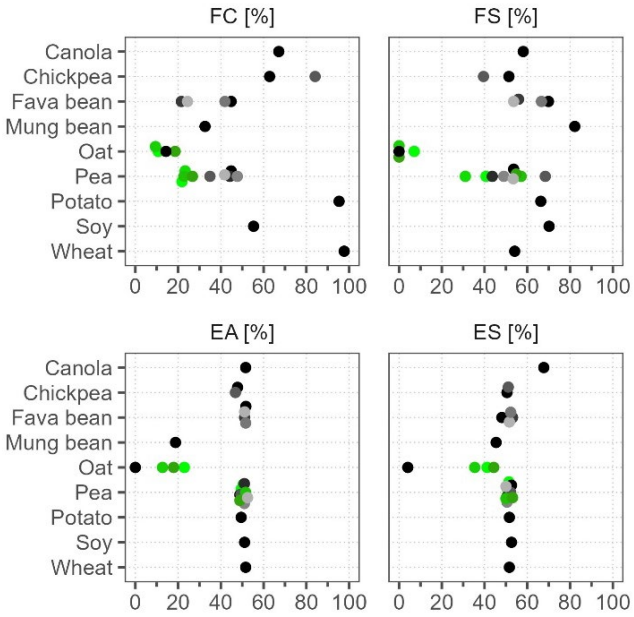

*Figure 8. Emulsifying and foaming properties of commercial plant protein powders. Each dot represents an individual product, while green symbols indicate different batches of the same product. FC–foaming capacity; FS–foaming stability; EA–emulsifying activity; ES–emulsifying stability (**Publication I**).*

Overall, ES values were similar to EA (48–53%) in most proteins, indicating that solubility alone does not determine emulsification performance. Batch-to-batch variation was minor in pea, but more pronounced in oat, although their low values limited practical relevance.

4.1.4 Sensory properties

As shown in Figure 9, nearly all protein powders had intense raw material–related odour and taste, with chickpea being the only sample without a distinct raw material odour, though still rated high in overall intensity. Chickpea, fava bean, mung bean, and canola exhibited the strongest odour and taste profiles, whereas soy, oat, wheat, and some pea samples were milder. Considerable variation was observed within pea proteins, with differences of up to 3.2 scoring units across batches. Off-flavours were generally low (<2.2 score), except for mung bean (rubbery/dusty odour, soapy/mouldy taste) and one pea batch (sulphurous odour).

Bitterness and astringency varied strongly by raw material, with fava bean generally more bitter and astringent, while oat scored the lowest.

Perception of particles was another important sensory aspect. Wheat, mung bean, and certain pea samples contained the largest particles, whereas oat and mung bean showed the greatest number of particles. In contrast, soy, canola, potato, and Pea 5 were smooth and scoring lowest for particle attributes.

Batch-to-batch variation was again most pronounced in pea proteins, with odour differences ranging from sulfuric and cheesy notes to sweeter, green-like nuances, likely reflecting differences in cultivar, growing conditions, and processing methods (García Arteaga et al., 2021; Vaikma et al., 2021).

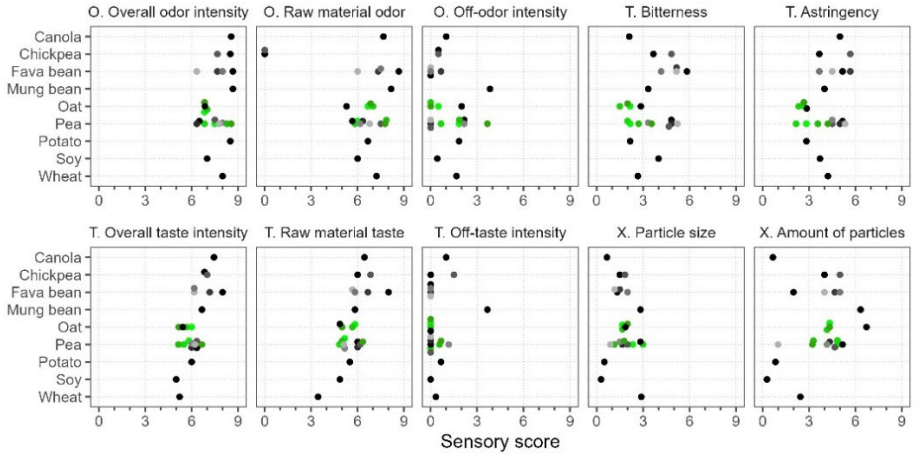

*Figure 9. Sensory evaluation of commercial plant protein powders. A 0–9 scale was used, defined as "none" to "very strong" for sensory attributes, "particles missing" to "very big" for particle size, and "particles missing" to "mostly particles" for particle amount. Each dot represents an individual product, while green symbols indicate different batches of the same product. O. –odour; T. –taste; X. –texture (**Publication I**).*

4.1.5 Relationships between physicochemical, techno-functional, and sensory properties

Figure 10 displays the statistically significant Spearman's rank correlation coefficients, multiplied by 100 for brevity. The results show that several techno-functional properties of plant protein powders are closely linked, indicating that certain ingredients can provide relatively broad functionality across different end-use applications. In particular, WHC, protein content, native pH, solubility at native pH, FC, and OHC tended to correlate, suggesting that powders with favourable water binding and appropriate solubility are more likely to perform well in both meat and dairy alternative systems.

Protein content was strongly associated with water and oil binding and with foam formation, confirming that proteins are the main contributors to these functionalities. However, protein content alone did not predict solubility, indicating that other components of the powder matrix, such as fibre or residual starch, also influence dispersibility and application behaviour. This highlights the limitation of relying solely on protein purity as a quality indicator for ingredient selection.

WHC emerged as a central practical indicator of functional performance. Powders with high WHC also showed good solubility at native pH and favourable interfacial properties, supporting their suitability for multiple applications. At the same time, high WHC was associated with reduced solubility under acidic conditions, suggesting that ingredients with strong water-binding capacity may aggregate more readily during

fermentation. This has direct implications for the formulation of fermented DA, where dispersion stability after acidification is critical.

Native pH influenced solubility behaviour after heat treatment, indicating that powders with higher native pH values are more likely to remain dispersed in neutral-pH products. As native pH is determined by the manufacturer during the isolation process, it represents a practical parameter that can be adjusted to tailor ingredients for specific applications. The different solubility measurements used in this work were not strongly correlated, demonstrating that solubility varies according to the analytical conditions applied and that the chosen test method should reflect the intended processing conditions in end applications.

Particle-related characteristics were inversely associated with key functional and sensory properties. Powders containing fewer and smaller particles exhibited better dispersibility behaviour, while undissolved particles contributed to the perception of granularity in liquid systems. This confirms that adequate solubility and controlled particle characteristics are essential for achieving acceptable mouthfeel in dairy alternative products.

These findings indicate that WHC, native pH, and solubility-related properties can serve as practical indicators for predicting broader techno-functional performance, while also emphasizing that functionality depends on the combined effects of composition, processing history, and physical structure. This provides a basis for more targeted ingredient selection and specification using a limited set of measurable parameters.

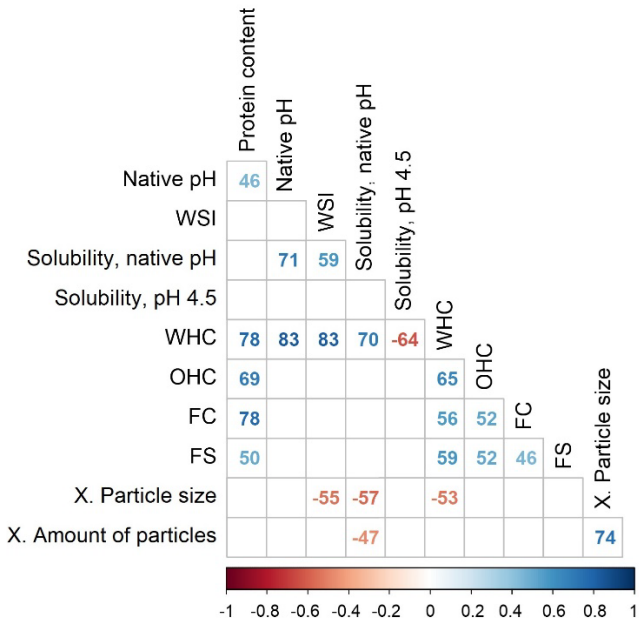

Figure 10. Spearman's rank correlations between the techno-functional properties of commercial plant protein powders. Correlation coefficients are multiplied by 100 for brevity. Only statistically significant correlations are shown (α = 0.05). WSI–water solubility index; WHC–water holding capacity; OHC–oil holding capacity; FC–foaming capacity; FS–foaming stability; G'–storage modulus; X. –sensory texture (adapted from ***Publication I****).*

Taken together, analysis of commercial plant protein powders revealed substantial variation in techno-functional and sensory properties. Solubility was generally low across samples, with only a few products showing high values. Heat treatment improved solubility in some cases, particularly at neutral pH, whereas acidic conditions typically reduced it. Notably, batch-to-batch differences were evident, especially in pea proteins, underlining challenges for reproducibility.

Water- and oil-holding capacities also differed considerably. In general, WHC was higher in powders with lower solubility, while OHC was relatively low and less variable across products. This trade-off indicates that poorly soluble proteins may be better suited for MA applications where juiciness is desired, while highly soluble powders are more appropriate for liquid or DA.

Foaming and emulsification properties showed no clear superior protein source. High FC did not necessarily translate into FS, and EA was moderate overall, with heat treatment only improving stability in selected cases. These findings confirm that interfacial properties are influenced by multiple factors beyond solubility alone.

Sensory evaluation further emphasized the limitations of current protein powders. Most products exhibited strong raw material-related odour and flavour, while off-flavours were less common but still problematic in some cases. Bitterness and astringency varied widely between sources, and pea proteins showed the greatest batch-to-batch variability, reflecting the influence of raw material origin and processing. Mouthfeel was strongly linked to solubility, with poorly soluble powders perceived as grainy and highly soluble ones as smooth. This negative correlation between solubility and textural granularity confirms that sufficient solubility is essential for successful application in DA, as undissolved particles otherwise cause sensory defects.

Overall, the results highlight solubility and sensory properties as the main challenges in the application of commercial plant protein powders. At the same time, the clear functional differences observed suggest application-specific opportunities: low-solubility, high-WHC powders may be better suited for MA, while highly soluble proteins hold greater potential for DA. However, overcoming strong inherent sensory attributes and reducing variability remain essential for improving consistency and consumer acceptance in plant-based products.

4.2 Influence of particle size and surface morphology of plant protein powders on the sensory perception of graininess in liquid matrices

In **Publication I**, most powders were only partially soluble and left undissolved material in aqueous dispersions that was frequently detected sensorially and contributed to grainy mouthfeel. Sensory assessment further indicated pronounced differences in the perceived size and amount of particles between samples, suggesting that dispersibility alone does not fully explain particle perception.

These findings highlight a critical challenge for liquid applications, such as DA, where smooth texture and dispersion stability are essential quality attributes. Undissolved particles in liquid matrices promote sedimentation and negatively affect mouthfeel. Despite existing research on particle properties, the sensory perception of plant protein particles under conditions relevant to dairy alternative production remains poorly understood.

Based on this knowledge gap, **Publication II** investigates the mechanisms governing sensory particle detection in plant protein dispersions. The study addresses why

dispersions that appear similar in terms of overall dispersibility may differ in perceived graininess. Particular emphasis was placed on the particles remaining in dispersion after processing, as these structures determine mouthfeel in the final product. A novel methodological approach was developed that combined instrumental PS analysis, sensory evaluation of perceived PS, and SEM-based assessment of particle morphology, enabling direct linkage between physical particle characteristics and sensory perception. By integrating instrumental and sensory data in heat-treated dispersions at native and acidified pH, this work provides a more comprehensive framework for evaluating plant protein powders with respect to their performance in smooth-textured liquid applications.

4.2.1 Method development for the assessment of particle morphology in plant protein water dispersions using SEM image visual analysis

We had demonstrated in **Publication I** that the sensory detection of particles in plant protein water dispersions could not be explained solely by their solubility behaviour. This indicated that additional structural features of the undissolved particles, such as shape and surface characteristics, contribute to mouthfeel. As plant protein powders are only partially soluble, the morphology of the particles remaining in liquid systems is directly relevant to the sensory quality of DA. Most previous studies have examined the morphology of spray-dried plant protein powders in the dry state, whereas the structure of particles persisting after hydration and heat treatment, conditions that determine mouthfeel in liquid products, has received little attention. Therefore, Publication II developed a dedicated methodological approach to visualize and evaluate the morphology of undissolved particles in realistic liquid systems.

Plant protein samples were prepared as 6% (w/w) aqueous dispersions, corresponding to protein levels typical of liquid dairy products and plant-based alternatives. The dispersions were heat-treated at 85 °C for 15 min and evaluated at both native pH and pH 4.5, representing conditions relevant to milk alternatives and fermented DA, respectively. These treatments were identical to those used for the solubility analyses of heat-treated plant protein dispersions in **Publication I** and for PS analyses. As most dispersions contained a fraction of non-dissolved material (Figure 11), the samples were mixed thoroughly prior to subsequent analyses to prevent stratification.

Figure 11. Example of the heat-treated plant protein dispersions in water after overnight storage at 4 °C.

To preserve the morphology of particles present in the liquid dispersions, a vitrification step was applied by rapid freezing in liquid nitrogen, followed by lyophilization. This procedure fixed the spatial structure of the undissolved particles and enabled their transfer to the SEM sample holder without collapse or aggregation. The freeze-dried material was mounted as a thin monolayer on carbon-taped SEM stubs, coated with a gold/palladium layer to improve conductivity, and imaged using environmental SEM. Images were recorded at two magnifications: ×200 to evaluate particle heterogeneity and ×500 to assess particle shape and surface roughness (Figure 12). Table 3 summarizes the sensory attributes, their definitions, and the scale

descriptions used for both the mouthfeel-based evaluation of perceived PA and the visual assessment of particle morphology.

*Table 3. Sensory attributes, their definitions, and the scale descriptions used for both the mouthfeel-based evaluation of perceived particle size and the visual assessment of particle morphology (**Publication II**).*

		Scale descriptions			
Attribute	**Definition**	**0**	**1**	**5**	**9**
Size	Describes the perceived size of particles. A protein solution was included as a reference. Evaluated based on mouthfeel.	Particles missing	Very small	Moderate	Very large
Roughness	Describes the un-evenness, coarseness of the particle surface. Photos included as references. Evaluated based on SEM images.	Completely smooth	Slightly rough	Half-smooth/ rough	Completely rough
Angularity	Describes the irregularity, angularity of particle shape. Photos included as references. Evaluated based on SEM images.	Completely circular	Slightly angular	Half-circular/ angular	Completely angular
Hetero-geneity	Describes how dissimilar are shapes and sizes of all particles. Photos included as references. Evaluated based on SEM images.	Completely homogenous	Slightly hetero-genous	Half-homogenous/ heterogenous	Completely hetero-genous

A novel aspect of this work was the development of a sensory-based visual assessment of SEM images to quantify particle morphology. Conventional software-based image analysis typically measures only two-dimensional contours and provides limited information on surface features. In contrast, trained assessors are able to infer three-dimensional particle characteristics from SEM images and provide more nuanced evaluations of surface roughness, angularity, and heterogeneity. A trained panel evaluated standardized SEM image sets displayed on a tablet, using predefined attribute definitions and anchored 10-point scales (0–9). Roughness described the degree of surface unevenness, angularity reflected the irregularity of particle shape, and heterogeneity represented the variation in size and shape within a sample. Two images per attribute were assessed to account for sample variability, and fibre-like fragments were excluded from evaluation.

The visual assessment protocol was developed through pre-evaluation sessions to ensure that attribute definitions captured the specific features observed in plant protein dispersions. This approach contributes to the limited body of literature on sensory evaluation of microscopic images and provides a structured framework for linking morphological features to sensory perception. In parallel, mouthfeel-based sensory evaluation of perceived PS and instrumental PSA were conducted on the same heat-treated dispersions, enabling correlation between morphology, measured particle characteristics, and oral perception.

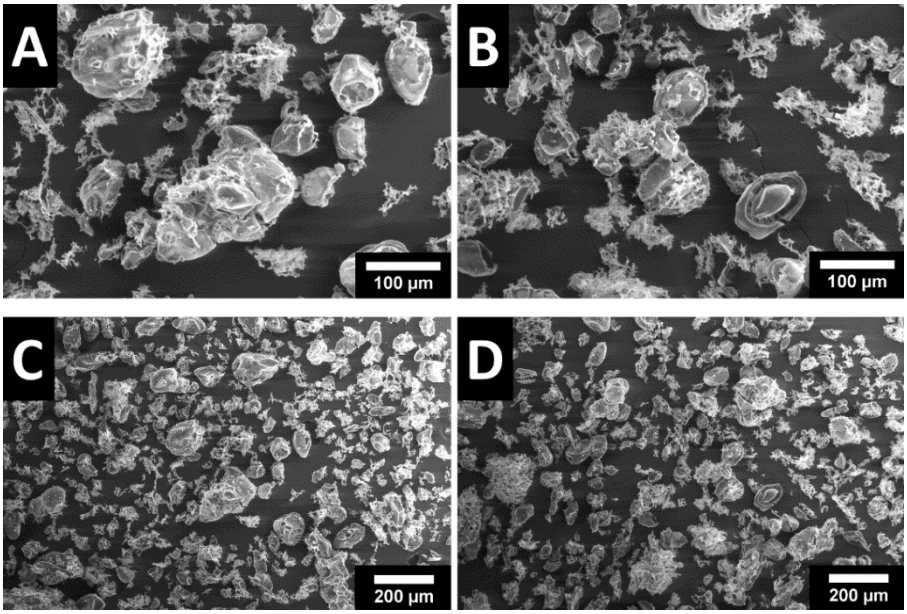

*Figure 12. Example of scanning electron microscopy image presentation for visual scoring. Images A and B (high magnification) and C and D (low magnification) were taken from two positions of a sample. The sample was a 6% water dispersion, heat-treated (85 °C, 15 min), acidified to pH 4.5, and lyophilized (**Publication II**).*

4.2.2 Particle size distribution

In the dry state, the average D90 of powders was 114 µm (range 56–235 µm), with large variation among pea and oat samples (Figure 13). Processing conditions such as spray drying or additional grinding accounted for much of this variation (Burger et al., 2022a).

When dispersed and heat-treated, PS increased considerably (Figure 13 and Table 4. At native pH, D90 values ranged from 75 µm to 375 µm, and at pH 4.5 from 73 µm to 493 µm. In about half of the samples, acidification had little effect, while in others it promoted aggregation. Soy and wheat formed visible aggregates of 1–2 mm, despite showing high solubility under some conditions.

These changes reflect swelling, partial dissolution, and aggregation driven by heat and pH. At pH 4.5, close to the isoelectric point of plant globulins, electrostatic repulsion is minimized and hydrophobic interactions favour large aggregate formation (Mession et al., 2013; Q. Tang et al., 2023; Yang et al., 2024). Variability was greater in liquid samples than in dry measurements, and correlations across states were weak ($\rho = 0.69$ between native and acidic pH, 0.18 between dry and liquid). This demonstrates that dry PS or native pH dispersion does not predict behaviour in acidic conditions and emphasizes the importance of conducting analyses under processing conditions that reflect the intended application to ensure reliable assessment of ingredient functionality in liquid plant-based products.

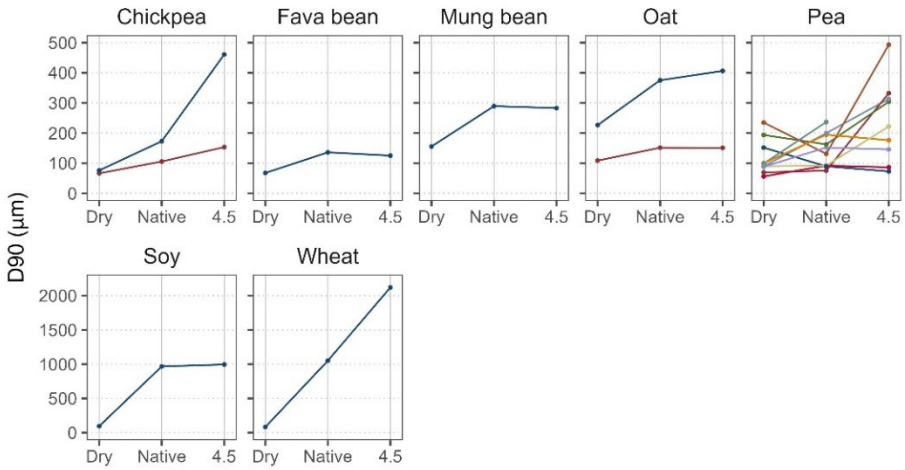

*Figure 13. Average volume-weighted particle diameters (D90) of plant protein powders measured instrumentally in the dry state and as heat-treated (85 °C for 15 minutes) 6% water dispersions at native pH and pH 4.5. Coloured lines represent different products from the same crop. Soy and wheat have different y-axis scale (**Publication II**).*

4.2.3 Sensory perception of grittiness

Sensorially perceived PS scores at native and acidified pH are summarized in Figure 14 and Table 4. Acidification generally amplified particle perception across the samples, even in cases where particles had previously been undetectable, most likely due to structural changes and aggregation. At native pH, perceived particle sizes ranged from no detection (score 0.0) to moderate-sized particles (6.1), while at pH 4.5 the range extended from very small particles (1.1) to very large particles (9.0). Pea samples demonstrated highly variable responses: for example, Pea9 increased from 0.0 to 5.8, whereas Pea1 remained nearly unchanged (1.3 to 1.1). The soy sample exhibited the most pronounced increase, from 0.0 at native pH to 8.4 after acidification. Interestingly, despite a large instrumental D90 of 968 µm at native pH, soy particles were not perceived, which may be explained by their hygroscopic nature: the powder formed soft, cloudy clumps that were likely softer than the oral mucosa and therefore undetectable (Appelqvist et al., 2015; Shewan et al., 2020).

These findings confirm that pH and heat treatment are critical in shaping sensory perception of PS in plant protein dispersions. Prior studies have shown that grittiness depends not only on PS but also on shape, hardness, concentration, viscosity, and matrix conditions (Imai et al., 1995; Livney et al., 1995; Olarte Mantilla et al., 2020; Petersson et al., 2013). In this publication, dispersion conditions were selected to reflect commercial DA. With plant protein powders typically showing low solubility (Jakobson et al., 2023), the resulting 6% particle concentration exceeded known detection thresholds, while the low viscosity of the dispersions (30 mPa s at 9% protein) provided little masking effect. For comparison, viscosity effects on perception have been reported only above 100 mPa s (Appelqvist et al., 2015). Instrumental particle sizes (D90 73–493 µm) were within or above detectable ranges, yet at native pH half of the samples had sensory scores below 0.5. This discrepancy suggests that factors beyond PS, such as particle softness or interactions with the matrix, also influence the perception of graininess.

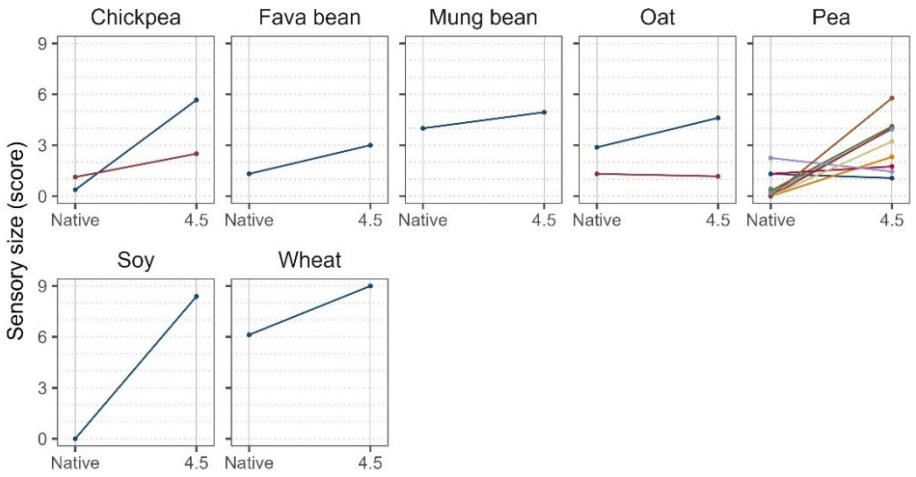

*Figure 14. Ladder plot showing the average particle size of plant protein powders assessed sensorially in 6% heat-treated (85 °C, 15 min) water dispersions at native pH and pH 4.5. Coloured lines represent different products from the same crop (**Publication II**).*

4.2.4 Relationship between particle size distribution and sensory perception

When sensory size was plotted against D90 in the liquid state, a clear but non-linear trend emerged Figure 15. A linear relationship appeared only at D90 values above ~200 µm, corresponding to sensory scores above 2, irrespective of pH. Below this threshold, no consistent trend was found. For example, fava bean at pH 4.5 and Pea9 at native pH both had D90 values near 130 µm, yet their sensory sizes differed markedly (score 3 versus undetectable). Similarly, Pea2 and Pea4 at native pH received scores of 0.0, despite D90 values of 75 µm and 195 µm, respectively.

The lack of correspondence for smaller particles suggests that factors beyond size determine sensory perception. Since variance in sensory scores did not increase for small particles, detection thresholds alone cannot explain the trend. Instead, physical properties such as hardness, deformability, solubility, water absorption, and surface morphology likely play a role. Supporting evidence comes from Appelqvist et al. (2015), who reported that soft carrot cell wall particles (30–400 µm) were undetectable in custards, and from Engelen et al. (2005), who found that round, soft polystyrene particles were less perceptible than rigid silica dioxide particles of similar size, with 80 µm silica dioxide perceived as larger than 230 µm polystyrene (Appelqvist et al., 2015; Engelen et al., 2005). Given the processing conditions applied, it is reasonable to assume that the plant protein particles in this publication were relatively soft. The absence of any association between powder solubility and D90 further justified examining particle morphology using SEM.

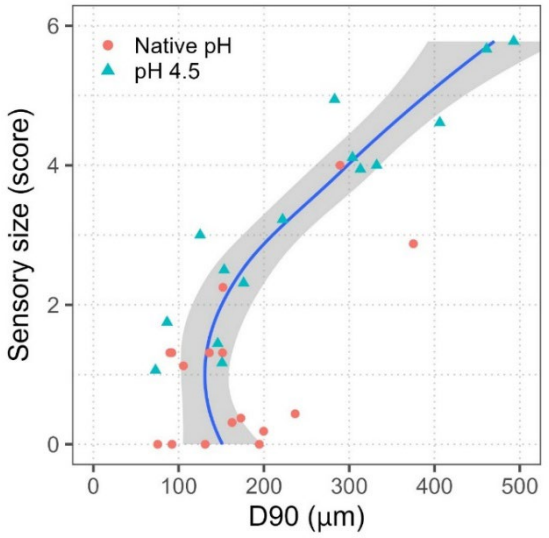

*Figure 15. Sensory perception of particle size as a function of the instrumentally measured volume-weighted particle size (D90) in 6% water dispersions of plant protein powders after heat treatment (85 °C for 15 min) at native pH and pH 4.5. The blue line represents the LOESS regression, and the gray band indicates the 95% confidence interval. Extreme values for soy and wheat were excluded (**Publication II**).*

4.2.5 Particle morphology analysis via visual assessment of SEM images

SEM images of selected samples are shown in Figure 16, with quantitative scores summarized in Table 4. Angularity described large-scale edges, roughness reflected fine surface features, and heterogeneity captured shape variation. Small amounts of dissolved dry matter were visible due to lyophilization (Beech et al., 2015), but were disregarded during evaluation.

At native pH, Pea4 had a large D90 but was not detected sensorially, corresponding to smooth and round particles with low roughness, angularity, and heterogeneity. After acidification, its sensory score increased, and particles appeared rougher and more angular. Pea6, despite a smaller D90, was detected sensorially and showed rough, angular particles. By contrast, Pea8 had a similar D90 but remained undetected, consistent with smooth and round morphology; after acidification, both sensory and visual scores increased sharply. These examples indicate that surface morphology, rather than size alone, determines whether particles are perceived.

Overall, the three visual attributes were highly intercorrelated (ρ = 0.75–0.97), but none correlated with D90 (ρ = 0.25–0.35). Thus, rough, angular, and heterogeneous particles were consistently more perceptible than smooth and round ones, regardless of their size.

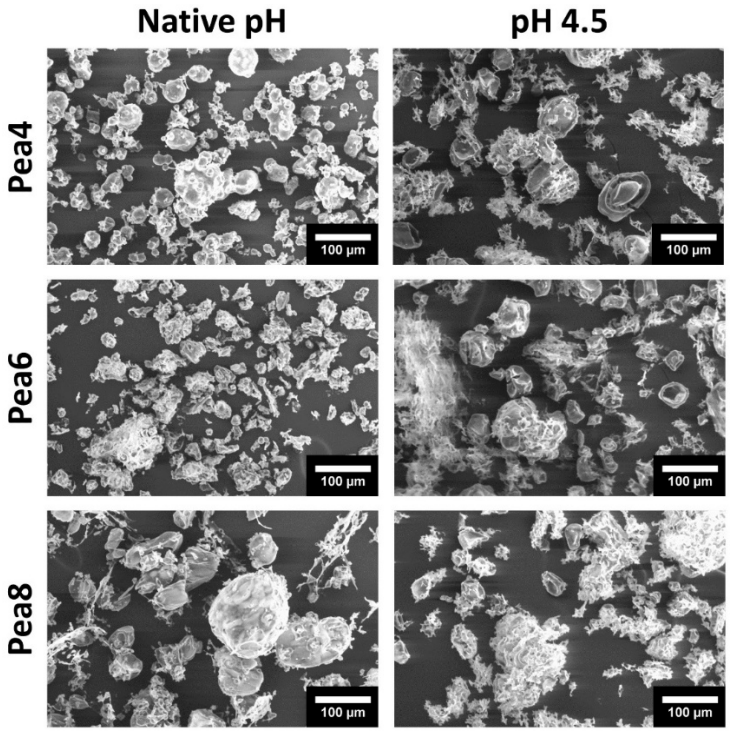

*Figure 16. Examples of scanning electron microscopy images of protein powders. The corresponding sensory scores are presented in Table 3. Powders were dispersed in water at 6% concentration, heat-treated at 85 °C for 15 min, subsequently maintained at native pH or adjusted to pH 4.5 and then lyophilized (**Publication II**).*

Taken together, the results showed that graininess in plant protein dispersions is influenced not only by PS but also by processing conditions and particle morphology. Acidification and heat treatment promoted aggregation near the isoelectric point, leading to large and variable particle sizes, yet sensory detection did not consistently follow instrumental D90 values. A threshold relationship was evident only above ~200 µm, while several samples with comparable sizes differed in detectability. SEM analysis demonstrated that rough, angular, and heterogeneous particles were more likely to be perceived than smooth and round ones. These findings suggest that both physicochemical transformations during processing and surface morphology must be considered when evaluating protein powders for liquid applications. Importantly, PSA under application-relevant conditions is essential, as dry-state measurements did not predict sensory outcomes.

*Table 4. Average sensory scores with standard deviations for particle morphology attributes evaluated from scanning electron microscopy images (10 assessors, two replicates). Corresponding sensory particle size evaluations (9 assessors, two replicates) and instrumentally measured particle sizes (10 replicates) are presented for comparison (**Publication II**).*

Sample	pH	Rough-ness	Angu-larity	Hetero-geneity	Sensory size	Instru-mental size D90, µm
Pea4	Native	2.9 ± 0.9	2.3 ± 1.0	2.8 ± 1.0	0.0 ± 0.0	195 ± 8
Pea8	Native	3.0 ± 0.7	2.8 ± 0.8	4.3 ± 0.9	0.0 ± 0.0	92 ± 2
Pea5	Native	4.3 ± 0.7	5.0 ± 0.9	4.8 ± 0.9	0.4 ± 0.4	237 ± 4
Pea1	4.5	6.5 ± 0.7	6.4 ± 0.8	4.0 ± 0.9	1.1 ± 0.2	73 ± 3
Oat2	4.5	7.7 ± 0.9	6.4 ± 1.0	7.9 ± 0.9	1.2 ± 0.3	151 ± 6
Pea6	Native	6.1 ± 0.7	6.3 ± 0.7	4.2 ± 0.9	1.3 ± 0.5	92 ± 1
Pea1	Native	8.0 ± 1.0	7.0 ± 0.8	7.3 ± 1.0	1.3 ± 0.6	90 ± 2
Oat2	Native	8.5 ± 0.7	8.2 ± 0.8	8.3 ± 0.7	1.3 ± 0.5	151 ± 10
Pea6	4.5	3.7 ± 0.6	4.5 ± 1.0	5.2 ± 0.9	1.8 ± 0.4	86 ± 2
Pea4	4.5	5.1 ± 0.9	4.5 ± 1.0	5.9 ± 1.0	2.3 ± 0.5	176 ± 18
Pea8	4.5	7.7 ± 0.9	6.6 ± 0.9	6.6 ± 0.5	3.2 ± 0.8	222 ± 33
Pea9	4.5	7.0 ± 0.8	6.1 ± 0.8	6.8 ± 1.0	5.8 ± 0.5	493 ± 54
Soy	4.5	8.2 ± 0.8	7.3 ± 0.7	6.9 ± 1.0	8.4 ± 0.5	996 ± 359
Wheat	4.5	8.8 ± 0.4	8.5 ± 0.7	8.2 ± 0.8	9.0 ± 0.0	2120 ± 618

4.3 Fermentation by lactic acid bacteria during pea protein isolation: improvement of sensory quality, and alteration of techno-functional properties

Plant protein powders often exhibit raw material–specific notes such as “pea-like,” grassy, bitter, and astringent flavours, which limit their use in alternative protein products and lower consumer acceptance. These undesirable off-notes originate from volatile aldehydes, alcohols, and ketones, as well as bitter and astringent compounds such as saponins and phenolics, and they remain one of the key barriers for the wider application of plant protein isolates in DA and MA. Thus, strategies for mitigating these flavours during ingredient processing are essential (Jakobson et al., 2023; Sarkar, 2024; Vaikma et al., 2022).

In Publication III, the aim was to explore lactic acid fermentation as a method for improving the sensory quality of pea protein isolates by reducing undesirable flavours and off-notes, while also evaluating its effect on techno-functional properties. Unlike conventional wet isolation, which relies on mineral acids for isoelectric precipitation, LAB can lower the pH biologically through fermentation, potentially replacing chemical acidification while simultaneously modifying flavour-active compounds.

To achieve this, five commercial starter cultures (SC1–SC5) were first screened for their ability to ferment pea protein solutions. All cultures reduced the pH to 4.8, but their fermentation kinetics and sensory impact varied. Based on preliminary screening, SC1 and SC2 were selected for further tests: SC1 for its fast acidification, and SC2 for its more pronounced reduction of pea-like odour and flavour identified by an informal sensory evaluation. Using these cultures, two fermented pea protein isolates (PPI_SC1 and PPI_SC2) were produced during the protein isolation process, alongside a chemically

acidified control (PPI_Ctrl). The protein solutions were fermented at 40 °C until reaching pH 4.8, followed by centrifugation, neutralization, and lyophilization. This design enabled direct comparison of fermented and chemically precipitated isolates in terms of sensory, physicochemical, and techno-functional properties.

The following subsections present the main findings on the sensory characteristics and techno-functional behaviour of the fermented pea protein isolates, and their implications for the development of plant-based protein ingredients.

4.3.1 Techno-functional properties

Fermentation by LAB altered several physicochemical and techno-functional properties of pea protein isolates (Table 5). WSI decreased by approximately 20% after fermentation, likely due to aggregation of protein particles at the isoelectric pH and prolonged exposure to elevated temperature, as well as possible interactions with LAB biomass or exopolysaccharides. Despite this decline, the WSI values of the fermented isolates remained higher than those reported for many commercial PPIs produced by spray-drying (Jakobson et al., 2023). In contrast, WHC nearly doubled in fermented isolates compared to the control, which can be explained by the inverse relationship between WSI and WHC.

OHC showed little change across treatments, with all isolates at ~1.7 g oil g^{-1}, a higher value than typical commercial PPIs (Jakobson et al., 2023), likely due to lyophilization. Protein surface hydrophobicity was not affected by fermentation, suggesting minimal changes to protein conformation.

FC improved markedly, particularly in PPI_SC2, reaching 83% compared to 45% in the control. FS, however, remained largely unchanged. This improvement is consistent with reduced solubility, which facilitates the formation of a more stable air–water interface. EA was unaffected, while ES decreased slightly after fermentation, consistent with unchanged surface hydrophobicity.

Overall, fermentation caused moderate changes to techno-functional properties, primarily reducing solubility but enhancing WHC and FC, with limited effects on emulsification and digestibility. These results indicate that fermentation can improve some functional properties while slightly compromising others, without fundamentally altering protein quality.

Table 5. Physicochemical, and techno-functional properties of pea protein isolates produced by fermentation with starter cultures (SC) compared with a chemically precipitated control (Control). Values are presented as mean ± standard deviation on a dry matter basis (analytical n = 3). Values within a row not sharing a letter differ significantly. The "Fermentation effect" row indicates the direction of change relative to the control, considering whether the effect was desirable or undesirable.

Property	Fermentation effect	Control	SC_1	SC_2
Water holding capacity, g H_2O g^{-1}	↑	0.78 ± 0.02^{a}	1.53 ± 0.02^{b}	1.68 ± 0.03^{c}
Oil holding capacity, g oil g^{-1}	↑	1.65 ± 0.02^{a}	1.66 ± 0.01^{ab}	1.70 ± 0.02^{b}
Water solubility index, %	↓	74.0 ± 1.4^{a}	55.8 ± 0.2^{b}	54.2 ± 0.5^{b}
Protein solubility, %	≈	37.0 ± 4.4^{a}	37.2 ± 1.0^{a}	38.2 ± 2.5^{a}
Foaming capacity, %	↑	45.5 ± 2.2^{c}	66.7 ± 1.1^{b}	83.3 ± 1.1^{a}
Emulsification activity, %	≈	37.2 ± 1.0^{a}	37.8 ± 1.0^{a}	38.3 ± 0.0^{a}

4.3.2 Sensory characteristics

The sensory evaluation revealed clear differences between the chemically precipitated control (PPI_Ctrl) and the fermented isolates (PPI_SC1 and PPI_SC2) (Figure 17). Fermentation significantly reduced pea-like odour and flavour, as well as overall flavour intensity, confirming the observations from preliminary experiments. For example, the pea-like odour score decreased from 7.4 in PPI_Ctrl to 5.6 and 4.4 in PPI_SC1 and PPI_SC2, respectively. Similar reductions were observed for pea flavour. This decrease coincided with a lower concentration of aldehydes, particularly hexanal and nonanal, which are major contributors to "green" and "pea-like" notes (Liu et al., 2023; Zhogoleva et al., 2023).

The most notable effect of fermentation was observed in bitterness, which decreased threefold in PPI_SC2 compared to PPI_Ctrl. As bitterness in peas is primarily caused by saponins, bitter peptides, and free fatty acids, fermentation likely reduced these compounds (García Arteaga et al., 2022; Gläser et al., 2021; Heng et al., 2006; Mittermeier-Kleßinger et al., 2021). Astringency was also reduced by about one score point, although the effect was less pronounced. This aligns with previous findings that fermentation can partly degrade phenolic compounds, which contribute to astringency, but cannot fully eliminate the sensation because protein–saliva interactions also play a role (Sarkar, 2024).

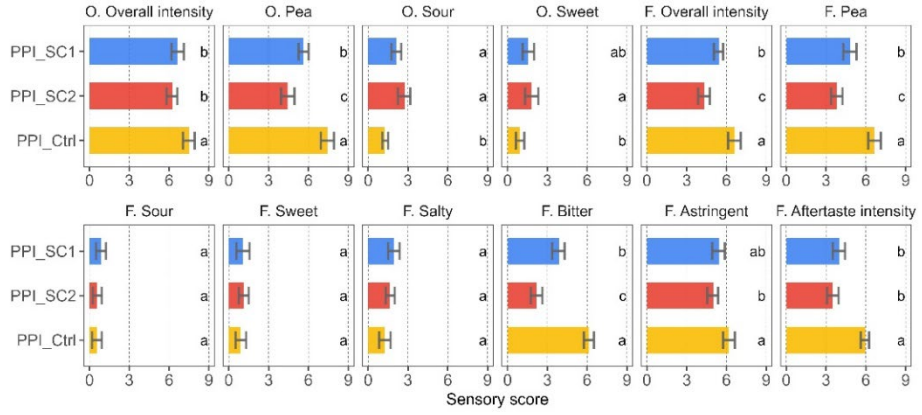

*Figure 17. Average sensory scores of pea protein isolates (PPI) produced using starter culture precipitation (SC) or chemical precipitation (Ctrl). Error bars indicate 95% confidence intervals (n = 16). O–odour; F–flavour (**Publication III**).*

Interestingly, PPI_SC2 exhibited the most favourable sensory profile, despite having higher total concentrations of aldehydes and alcohols compared to PPI_SC1 (Table 3). This discrepancy may be explained by differences in pyrazine content, as well as possible masking effects of certain volatiles (Ben-Harb et al., 2019). In addition to reducing undesirable notes, fermentation slightly enhanced sweet and sour odours, which can be linked to the formation of acetic and hexanoic acids, as well as fruity aldehydes such as benzaldehyde and branched-chain butanals.

Overall, fermentation by LAB during pea protein isolation effectively replaced chemical acidification and markedly improved the sensory quality of pea protein isolates. LAB fermentation substantially reduced raw-material-specific flavours, especially pea-like notes and bitterness, without introducing negative sensory attributes. At the same time, techno-functional properties were only moderately affected: solubility decreased,

but WHC and FC increased, while emulsifying properties were only slightly altered. The findings highlight the potential of fermentation during isolation to expand the use of pea protein in alternative dairy and meat applications.

4.4 Effect of extrusion processing parameters on the techno-functional, textural, and sensory properties of plant-based meat alternatives

The techno-functional properties of plant protein powders vary widely across sources, and in some cases remain insufficient for their successful application in alternative protein products. Extrusion is a key technology to overcome these limitations, as it can fundamentally modify protein structure and functionality, enabling the production of TVP that mimic the texture and sensory properties of meat. LME in particular is widely employed for MA due to its cost-effectiveness, high productivity, and ability to generate fibrous structures through protein denaturation and alignment under heat and shear (Kaleda et al., 2021; Sman & Goot, 2023). However, the outcome is highly dependent on process parameters such as moisture content, screw speed, and pH, which determine protein interactions, water binding, and ultimately the texture and acceptability of the product.

In **Publication IV**, an upcycled raw material, dry-fractionated DWMP, was introduced into TVP formulations alongside pea protein isolate (75:25). DWMP represents a sustainable, protein-rich by-product from semolina production, which has potential for valorisation in alternative protein applications. Using a reduced factorial experimental design, we systematically investigated the influence of pH (6.9 vs. 7.5), screw speed (400 vs. 600 rpm), and moisture content (28% vs. 32%) on the physicochemical, techno-functional, textural, and sensory properties of the extrudates. By adjusting these processing variables, the aim was to explore how extrusion can be employed to tailor the quality of plant-based MA and to determine whether innovative protein sources such as DWMP can deliver products comparable to those produced from conventional ingredients.

The following subsections present the results of this work, first focusing on the techno-functional and textural properties of the extrudates, and then on their sensory evaluation.

4.4.1 Techno-functional and sensorially perceived texture properties of the extrudates

The response surface models for the physical and sensorially perceived texture properties of the extrudates that were significantly affected by the extrusion parameters are presented in Figure 18. Water holding capacity (WHC) of whole extrudates increased with screw speed, most clearly at pH 7.5, consistent with higher expansion and porosity that promote water entrapment. Elevating pH from 6.9 to 7.5 favoured higher WHC in these samples. Further, as the pH was increased during extrusion, sensorially perceived moistness increased, whereas sensorially perceived hardness decreased. Perceived fibrousness, a critical attribute for enabling TVP to mimic the fibrous structure of meat, was significantly influenced by increased moisture during extrusion and a moisture × screw speed interaction suggested that fibrousness can be fine-tuned by balancing these two settings. This aligned with higher WHC and sensory moistness at elevated pH, indicating that better water retention translated into softer, juicier perception during chewing.

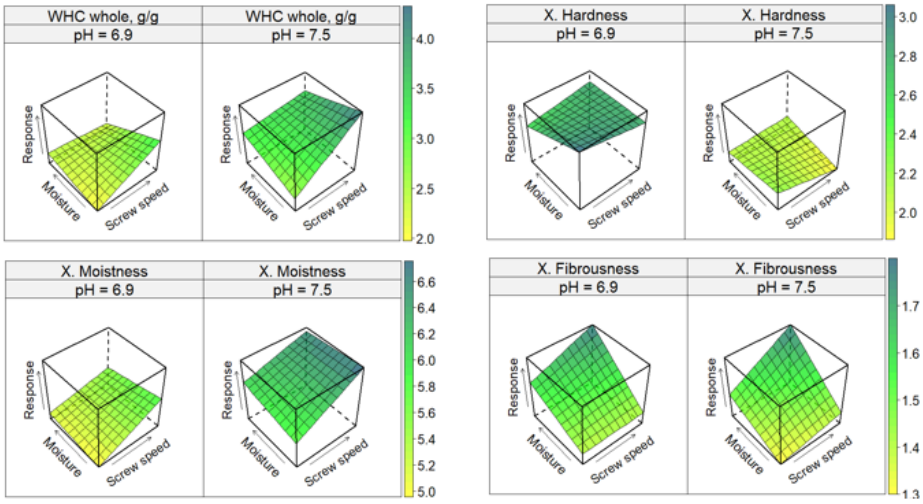

*Figure 18. Response surface models relating changes in pH, screw speed, and moisture content to water holding capacity (WHC), and sensory hardness, moistness, and fibrousness of the extrudates. X. –sensory texture (**Publication IV**).*

4.4.2 Odour and flavour properties of the extrudates

Figure 19 summarizes the differences in all measured sensory attributes across the study samples. The texture-related attributes that were significantly affected have been discussed in Section 4.4.1. As illustrated by the violin plots, perceived moistness and hardness exhibited the greatest variability among the samples, indicating that these attributes were most strongly influenced by the extrusion conditions. In contrast, the odour and flavour attributes of the rehydrated extrudates were not significantly affected by the processing parameters. In particular, bitterness and astringency, commonly reported as undesirable characteristics in plant-based protein products, remained unchanged across the tested combinations of pH, moisture content, and screw speed. This indicates that the incorporation of DWMP and the applied processing conditions did not introduce additional off-flavours compared to the reference formulations.

The absence of process-induced flavour differences suggests that the modifications in extrusion parameters primarily influenced structural properties rather than flavour generation. This is technologically relevant, as flavour stability during texturization allows optimization of texture and juiciness without the need for additional flavour-masking strategies. It also confirms that the use of DWMP as a partial replacement for pea protein isolate does not negatively affect the sensory flavour profile of the resulting TVP.

These results demonstrate that extrusion parameter adjustments can be used to tailor textural attributes while maintaining a neutral flavour profile, which is essential for the subsequent seasoning and formulation steps typical of meat alternative product development.

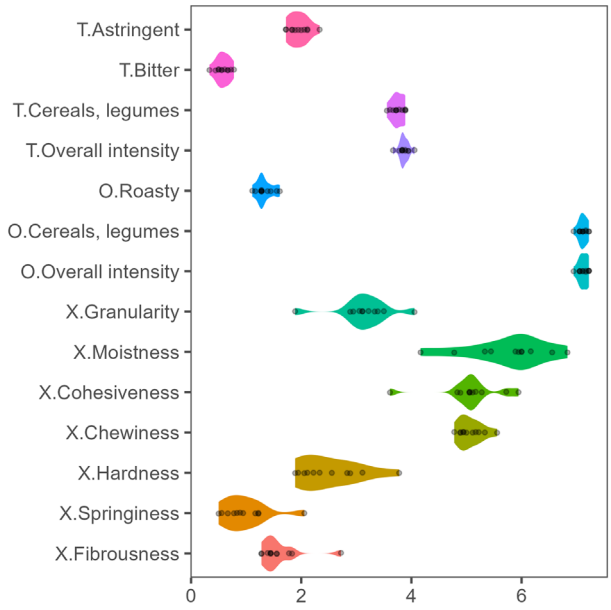

Figure 19. Violin plot summarizing the distribution of sensory scores for all measured sensory attributes across the study samples. Sensory scores are presented on a 0–9 scale on the horizontal axis. O. –odour; T. –taste; X. –texture.

4.4.3 Comparison with reference TVP

To evaluate whether the pea–DWMP formulations could achieve properties comparable to established market products, two reference extrudates were produced: a pure pea protein isolate TVP (PPI), representing a widely used market product, and a pea protein isolate–pea starch concentrate blend (PPI_PSC), reflecting formulations in which proteins are combined with polysaccharides to enhance texture.

The sensory comparison showed that the texture of the DWMP-based TVP could be adjusted to match the range defined by these commercial references. The box plot on Figure 20 illustrates the sensory texture properties of the investigational study samples compared to reference. Perceived fibrousness of all experimental samples was comparable to the PPI reference, indicating that the incorporation of DWMP did not impair the formation of a fibrous, meat-like structure. In contrast, perceived hardness and moistness were generally closer to the PPI_PSC benchmark, reflecting the higher water retention achieved under specific processing conditions.

Extrusion parameters provided effective control over these attributes. Higher screw speed and elevated pH promoted softer and moister textures, while lower screw speed resulted in firmer products. Moisture content primarily influenced fibrousness, enabling modulation of this key structural attribute without compromising overall texture. These results demonstrate that DWMP can be incorporated as an alternative protein source while maintaining sensory properties comparable to commercial TVP. The extrusion parameters provide practical control points for tailoring hardness, moistness, and fibrousness to match target product characteristics.

Figure 20. Box plot of the sensory texture properties of the investigational study samples compared to reference texturized vegetable proteins. PPI= pea protein isolate; PPI_PSC= mix with pea protein isolate and pea starch concentrate; PPI_DWC = a blend of pea protein isolate and durum wheat cake, extruded under varying processing parameters.

This study demonstrated that screw speed, feed moisture, and pH are the primary processing variables governing the structure, water-binding behaviour, and sensory texture of pea–DWMP TVP. Screw speed had the strongest influence on expansion, WHC, and hardness, with higher screw speed producing softer and more hydrated structures. In contrast, increased feed moisture promoted the formation of a denser and more fibrous network, leading to enhanced perceived fibrousness but higher instrumental firmness. Adjustment of pH to 7.5 improved water retention and increased sensory moistness while reducing perceived hardness, highlighting the role of protein charge state in texture development under LME conditions.

Overall, textural attributes were primarily controlled by screw speed and moisture content, whereas pH acted as a modifier of hydration and sensory softness. Importantly, the incorporation of DWMP did not hinder fibrous structure formation and allowed the production of extrudates with sensory properties comparable to commercial pea-based references when appropriate processing conditions were applied.

These findings demonstrate that targeted control of extrusion parameters enables the tailoring of hardness, moistness, and fibrousness to meet desired product profiles while facilitating the use of an upcycled wheat side-stream ingredient. The results provide a practical framework for optimizing LME processes and support the valorisation of alternative protein sources in plant-based meat alternative applications.

4.5 Overall relevance, limitations, and future perspectives

The four studies presented in this dissertation address complementary challenges related to the functionality and sensory performance of plant protein ingredients, beginning with the analysis of market-level variability and extending to targeted modifications at the ingredient and processing levels.

Publication I establishes the foundation by mapping the functional and sensory variability of commercially available plant protein powders. By analysing multiple products and batches, the publication demonstrates that inter-batch variability, particularly pronounced for pea protein, is a defining characteristic rather than an exception. Native pH, WHC, and WSI were identified as simple, robust indicators of

functional performance, offering practical tools for improved ingredient specification and communication between suppliers and manufacturers. Importantly, this publication also introduces a comprehensive sensory characterization of plant protein powders, revealing that bitterness, raw-material-specific notes, and particle perception issues are already present at the ingredient level.

Building directly on these findings, **Publication II** focuses on particle perception in liquid systems, a critical challenge for dairy alternative applications. While PS was shown to influence oral detection, this relationship broke down at smaller size ranges. By utilizing a SEM image-based visual assessment of particle morphology, the publication demonstrates that shape, surface roughness, and heterogeneity are decisive factors for mouthfeel. This work extends the sensory insights of **Publication I** and provides a new methodological framework for selecting and designing plant protein ingredients for liquid applications.

While **Publications I** and **II** highlight inherent sensory challenges, **Publication III** explores fermentation as a targeted strategy to improve flavour quality at the ingredient level. By integrating lactic acid fermentation directly into the protein isolation process, the work achieved significant reductions in bitterness and raw-material-specific off-notes without major losses in techno-functional performance. The starter-culture-dependent effects observed demonstrate that fermentation outcomes can be tailored, while also underscoring that sensory optimization alone does not necessarily resolve functional limitations.

Addressing these functional challenges, **Publication IV** investigates LME as a processing-based solution for meat alternative applications. Through controlled adjustment of extrusion parameters, the publication demonstrates that diverse textural and functional properties comparable to commercial TVP can be achieved with minimal sensory compromise. This highlights the potential of process-driven modification and alternative raw materials to overcome functional constraints identified earlier in the dissertation.

Across all four studies, solubility emerges as a cross-cutting but context-dependent concept. The lack of direct correlation between solubility measures reinforces that solubility cannot be treated as a single intrinsic property, but rather as behaviour shaped by processing history and use conditions. Equally central is the consistent integration of sensory evaluation across ingredient and targeted product types, encompassing untreated, fermented, heat-treated, acidified, and extruded matrices. This integrated sensory–functional perspective represents a key contribution of the dissertation and provides a framework for more application-relevant plant protein research.

This dissertation has several limitations. It does not directly investigate ready-to-eat end products, nor does it address rheological behaviour, nutritional quality, digestibility, or the role of protein primary and secondary structure in determining functionality. These areas represent important directions for future research. Furthermore, progress in improving industrial plant protein powders is constrained by limited access to information on isolation and drying parameters due to intellectual property restrictions.

Future research should focus on harmonized and validated characterization methods, exploration of less refined protein ingredients, sustainable side streams, bioactive compounds, fibre–protein interactions, and hybrid protein solutions.

5 Conclusions

The aim of this dissertation was to investigate the techno-functional and sensory properties of commercially available plant protein ingredients and to identify strategies, through ingredient selection and processing, that enhance their suitability for application in alternative meat and dairy products.

1. Techno-functional properties of protein powders studied vary substantially, both between products from different crops and products derived from the same source, reflecting the strong influence of processing conditions in addition to raw materials.
    - Proteins differed markedly in properties such as solubility, WHC and OHC, and emulsifying and foaming behaviour, indicating their varying suitability for different food applications.
    - Solubility was generally limited and decreased further under acidic conditions typical of fermented products, often resulting in dispersions containing undissolved particles.
    - Many materials exhibited pronounced raw-material-specific odours and off-flavours, which may restrict their use in products requiring mild sensory profiles and highlight the need for strategies to reduce undesirable sensory characteristics.
    - Soy protein isolate showed the most favourable set of overall properties, while alternatives to soy proteins offered advantages in specific functionalities, including for example oil absorption (canola) and solubility under acidic conditions (some pea and fava bean).
2. Plant-based protein powder PS measurement in the dry state does not predict the sensory perception of particle size in their aqueous dispersions. Particle perception in the aqueous dispersions is influenced by both PS and surface morphology, with increased surface roughness contributing to the sensory perception of smaller particles.
    - The relationship between the sensorially perceived PS and instrumentally measured PS in plant protein powders dispersed in water was linear for particles bigger than 200 µm, with increasing PS corresponding to increased sensorially perceived PS.
    - Particles smaller than 200 µm can be sensorially detected when exhibiting higher surface roughness and angularity, whereas smooth and round particles of the same size may not be sensorially detected.
    - By developing a unique method combining SEM imaging with sensory panel-based visual grading to assess particle surface properties, the approach not only provided quantitative descriptors of particle morphology but also advanced methodologies for the sensory evaluation of visual data.
3. Lactic acid fermentation can be used to acidify and precipitate solubilized pea protein, thereby replacing chemical acidification in the conventional isolation process and reducing undesirable plant-protein odours, off-flavours, and bitterness, while having a smaller, starter culture-dependent effect on techno-functional properties.
    - Fermentation greatly reduces the undesirable flavours like bitterness, aftertaste intensity, pea odour, and taste.

- Starter culture choice has a significant impact on the sensory and some techno-functional properties.

4. Extrusion parameters have significant impact on the resulting TVP's structure and texture, while having limited impact on flavour and odour.
    - Higher screw speed enhances WHC while reducing WSI and instrumental hardness.
    - Higher feed moisture decreases WHC, increases hardness and springiness, and promotes a more fibrous structure.
    - Increased pH enhances WHC and sensory moistness, while reducing sensory hardness.
    - Processing parameters had no significant effect on the odour and flavour of the rehydrated extrudates.

To our knowledge, this work represents one of the first systematic comparisons of commercially available plant protein powders produced using different technologies across multiple botanical sources, characterized using a broad set of application-relevant methods combined with sensory analysis. Summarizing, the results of the current study show that, alongside botanical origin, processing conditions strongly contribute to the determination of the techno-functional properties of plant protein ingredients.

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Acknowledgements

This work was funded by the European Regional Development Fund (ERDF) and the Estonian Research Council through project RESTA16. The experimental work was carried out at TFTAK.

I would like to express my deep gratitude to my supervisors, Raivo Vilu, Tiina Kriščiūnaitė, and Aleksei Kaleda, for their exceptional guidance and support. Their knowledge, experience, thoroughness, and high standards have shaped this work profoundly. I can say with full confidence that throughout these years I have benefited from outstanding supervision. In every challenge and question, I have been able to rely on their expertise, advice, and encouragement.

I would also like to express my appreciation to my boss and mentor, Sirli Rosenvald, for her support, guidance and trust. Her clarity of thought, effectiveness, and strong sense of purpose have been a great source of inspiration. I respect these qualities and aim to carry them forward in my own professional development.

My thanks also go to my team, the diverse and talented Alternative Proteins group. It is a remarkable mix of people with different backgrounds and perspectives, whose ideas, experiences, and approaches have continually challenged me to grow and step beyond my comfort zone.

I am grateful to TFTAK's sensory team, who have played a central role in the professional evaluation of samples across my publications, and to the analytical team for their dedication and collaborative spirit. My sincere thanks go to the management team, with whom I have had the opportunity to contribute to the development of TFTAK. I would also like to thank my TFTAK colleagues, talented, open-minded, and inspiring people who make it a place where one can grow, learn, and truly realise one's potential.

Beyond TFTAK, I would also like to extend my heartfelt thanks to the professors at TalTech from different departments with whom I have had the opportunity to work during my doctoral studies, and especially those from the Department of Chemistry and Biotechnology, as well as the study and administrative support staff of the School of Science, who have always been very kind, supportive, and helpful. Both academic and social interactions with them have been consistently inspiring and uplifting.

Through my doctoral work, I have had the opportunity to build close and meaningful collaborations with academics from the Department of Soil, Plant and Food Science at the University of Bari. I am grateful for the expertise, support, and lasting connections that have grown from this collaboration. In particular, I would like to thank Vittoria Latrofa for playing an important role in my doctoral journey and for introducing extrusion into my PhD work.

I am especially grateful to all my co-authors, listed here in alphabetical order, for their valuable contributions to this research: Karl Adra, Francesco Caponio, Davide De Angelis, Aleksei Kaleda, Tiina Kriščiūnaitė, Vittoria Latrofa, Antonella Pasqualone, Sirli Rosenvald, Andres Sats, Navratan Sharma, Giacomo Squeo, Irina Stulova, Carmine Summo, Mari-Liis Tammik, Helen Vaikma, and Raivo Vilu.

I would like to express my sincere gratitude to Professor Emeritus Ruta Galoburda and Professor Marilyn Rayner for serving as opponents for this dissertation and for dedicating their valuable time and expertise to its evaluation. I am also very grateful to the members of the defence committee, listed here in alphabetical order: Dr. Kaarel Adamberg, Dr. Marie Kriisa, Dr. Mary-Liis Kütt, and Professor Katrin Laos, for their time, expertise, and valuable contributions to the assessment of this dissertation.

On a personal note, I am deeply grateful to my family, whose support has made it possible for me to live and study abroad, build friendships around the world, and remain mindful of what is truly important in life.

I would also like to thank my husband, my closest companion, with whom I have grown immensely during this PhD journey, both as an individual and as a partner. His support and presence have meant a great deal to me during this journey.

I also gratefully acknowledge the Estonian Doctoral School for Natural and Agricultural Sciences for supporting my participation in the workshop Reimagining Biotech & Food Science. I am thankful to Kristel Vene for organizing this inspiring event and to Yoad Mick for introducing me to the concepts of Proactive Imagination and the IMAGYM approach, whose emphasis on possibility-oriented thinking broadened my perspective and provided valuable inspiration during the final phase of my doctoral studies.

I would also like to express my deep appreciation to the Estonian Chamber of Commerce and Industry, and especially to Piret Potisepp, for creating the opportunity to participate in the Women Leaders Accelerator. The programme has provided valuable professional insight and the chance to learn from outstanding leaders in their field, while also serving as a source of motivation, encouragement, and energy during an intense period of my doctoral journey. Equally inspiring has been the group of participants, a remarkable community of women leaders from whom I have learned, grown, and shared this experience.

To sum up, I highly value the opportunity for doctoral students to take courses across different faculties and broaden their knowledge beyond their main field of study. This flexibility, together with the courses, collaborations, and connections throughout my PhD journey, has shaped my professional path and brought me to where I am today. For me, doctoral studies have been an ambitious and meaningful process of self-development in every sense, and I truly appreciate the experiences and relationships gained along the way.

Thank you, Alma Mater, Tallinn University of Technology. I carry these lessons forward in my academic and professional work, shaped by curiosity and a desire to learn.

Abstract

Physicochemical, Techno-Functional, and Sensory Properties of Plant Protein Food Ingredients and Their Technological Modification

As demand for sustainable protein sources grows, plant proteins have become central to the development of alternative meat and dairy products. Their integration into everyday diets is increasingly topical due to environmental, health, and ethical concerns surrounding conventional livestock production. However, compared with animal proteins, plant proteins generally exhibit lower nutritional quality and less favourable functional and sensory properties, which limits their applicability in diverse food formulations. The physicochemical, techno-functional, and especially sensory properties of commercial plant proteins also remain insufficiently characterized. The aim of this dissertation was therefore to systematically evaluate the techno-functional and sensory properties of commercial plant protein powders from diverse sources and to investigate modification strategies, including fermentation and extrusion, to improve their techno-functional and sensory characteristics relevant to alternative protein products.

Protein powders from pea, oat, fava bean, chickpea, mung bean, potato, canola, soy, and wheat were characterized for solubility, emulsification and foaming, water and oil holding capacity, and sensory attributes. Properties varied considerably both between crops and between batches of the same product, highlighting the strong influence of raw material and production conditions. Solubility was generally low, which constrains related properties such as emulsification and foaming. Moreover, solubility was particularly reduced under acidic conditions representative of fermented foods, thereby posing major challenges for liquid dairy alternatives, where particle-related texture nuances are especially critical. In these applications, mouthfeel constitutes a key determinant of quality and is closely linked to solubility, particle size, and surface morphology. Sensory evaluation showed that grainy texture depended not only on particle size but also on surface roughness and angularity. While particles above 200 µm correlated with sensory detection, perception below this threshold was governed by morphology: smaller rough and angular particles were perceptible, whereas smoother but somewhat larger ones were not. These results provide new insights into how non-dissolved particles contribute to the gritty perception often reported in plant-based dairy alternatives.

To address undesirable sensory characteristics such as bitterness, beany notes, and raw-material-specific flavours, fermentation with lactic acid bacteria during isolation of pea protein was successfully applied. This process markedly reduced bitter and raw material specific notes without compromising functional attributes, demonstrating its potential as a targeted strategy for producing more palatable protein ingredients. Extrusion was employed as a processing method to restructure and direct the texture of plant proteins for meat alternative applications. Processing parameters strongly influenced structural and textural attributes: increasing the pH from 6.9 to 7.5 enhanced moistness, increasing screw speed from 400 to 600 rpm reduced hardness, and raising moisture content from 28% to 32% promoted fibrous structure formation. These results confirm that extrusion conditions can be tuned to modulate the properties of texturized vegetable proteins, thereby tailoring product properties to consumer expectations.

Overall, this dissertation shows that both ingredient selection and targeted processing strategies can be leveraged to improve the techno-functional performance and sensory quality of plant proteins. The novelty of this work lies in its systematic evaluation of commercial protein powders under industrially relevant conditions combined with comprehensive sensory analysis, which remains rare in previous studies. The results contribute theoretical and practical insights into the links between physicochemical properties and sensory outcomes and demonstrate the potential of fermentation and extrusion to enhance the performance of plant proteins in sustainable and palatable dairy and meat alternatives.

Lühikokkuvõte

Taimsete valgupulbrite füüsikalis-keemilised, tehnofunktsionaalsed ja sensoorsed omadused toidurakendustes ning nende tehnoloogiline modifitseerimine

Taimsete valkude tähtsus alternatiivsete liha- ja piimatoodete arendamisel on üha kasvamas, mis on seotud loomakasvatusega seotud keskkonna-, tervise- ja eetiliste probleemidega. Loomse päritoluga valkude tootmine on ressursimahukas, mõjutab kliimat ning kurnab maa- ja veevarusid. Taimsetel valkudel on aga mitmeid piiranguid võrreldes loomsete valkudega: nende toiteväärtus on sageli madalam ning füüsikalis-keemilised, tehnofunktsionaalsed ja sensoorsed omadused kehvemad. Lisaks on tööstuslikult toodetud taimsete valgupulbrite omadusi seni ebapiisavalt uuritud. Käesoleva doktoritöö eesmärk oli uurida erinevatest taimsetest toorainetest pärinevate kommertsiaalsete valgupulbrite tehnofunktsionaalseid ja sensoorseid omadusi ning hinnata fermentatsiooni ja ekstrudeerimise kui modifitseerimisstrateegiate mõju omadustele, mis on olulised alternatiivsete liha- ja piimatoodete arendamisel.

Töös karakteriseeriti herne-, kaera-, põldoa-, kikerherne, mungoa, kartuli-, rapsi-, soja- ja nisuvalgu pulbreid, hinnates nende lahustuvust, emulgeerimis- ja vahustamisomadusi, vee- ja õlisidumisvõimet ning sensoorseid omadusi. Uuritud kommertsiaalsed valgupulbrid erinesid üksteisest märkimisväärselt nii taimeliikide, sama taimeliigi erinevate toodete kui sama toote eri partiide vahel, mis rõhutab nii tooraine ja tootmistingimuste olulisust. Pulbrite lahustuvus oli üldjuhul madal, mis omakorda mõjutas teisi laustuvusega seotud funktsionaalseid omadusi nagu emulgeerimis- ja vahutamisvõime. Drastiliselt vähenes toodete lahustuvus happelistes tingimustes (iseloomulik fermenteeritud toodetele), mis vähendab nende kasutusvõimalusi vedelates lõpptoodetes, kus osakestega tunnetusega seotud tekstuurinüansid on kriitilise tähtsusega. Vedelates toodetes on suutunnetus kõige olulisem kvaliteeditegur, mis on tihedalt seotud seotud lahustuvuse, osakeste suuruse ja -pinnamorfoloogiaga. Sensoorse hindamise käigus selgus, et teraline või jahune tekstuur ei tulene ainult osakeste suurusest, vaid ka nende pinnakaredusest ja nurgelisusest. Kui üle 200 µm-ste osakeste suurus korreleerus nende sensoorse tunnetusega (suuremaid osakesi tunnetati suuremana ka sensoorselt), siis sellest väärtusest väiksemate osakeste puhul määras tajumise ära osakeste morfoloogia: väiksemaid kareda ja nurgelise pinnaga osakesi tajuti, samas kui siledaid aga suuremad osakesed ei tajutud. Eespool mainitud tulemused annavad olulist uut teavet mittelahustunud osakeste rollist liivase tuutunde kujunemisel, mida taimsete piimaalternatiivide puhul sageli raporteeritakse.

Mittesoovitud sensoorsete omaduste (nagu kibedus, kaunviljadele iseloomulikud noodid ja toorainespetsiifilised kõrvalmaitsed) vähendamiseks kasutati hernevalgu isoleerimisprotsessi käigus edukalt piimhappebakteritega fermenteerimist. Uuritud protsess vähendas märgatavalt kibedaid ning tooraine-spetiifilisi kõrvalmaitseid samal ajal funktsionaalseid omadusi kahjustamata, tõestades selle tehnoloogia potentsiaali sihipärase strateegiana parandatud maitseomadustega taimsete valgupulbrite tootmiseks. Lihaanaloogide tekstuuri parandamiseks uuriti ekstrudeerimist, mis võimaldab taimsete valkude restruktureerimist kiulise struktuuri tekitamiseks. Töötlusparameetrid mõjutasid tugevalt struktuurseid ja tekstuuri omadusi: pH

suurendamine 6,9-lt 7,5-le suurendas toote niiskustunnet, kruvi pöörlemiskiiruse tõstmine 400-lt 600 rpm-ile vähendas kõvadust ning niiskusesisalduse tõstmine 28%-lt 32%-le soodustas kiulise struktuuri teket. Saadud tulemused kinnitavad, et ekstrudeerimisparameetreid saab sihipäraselt kohandada, et mõjutada tekstureeritud taimsete valkude vee sidumisvõimet, kiulisust, niiskust ja kõvadust ning seeläbi suunata toodete omadusi vastavalt lõpprakendusele ja tarbijate ootustele.

Kokkuvõttes näitab käesolev doktoritöö et nii tooraine valik kui sihipärased töötlusstrateegiad võimaldavad parandada taimsete valkude tehnofunktsionaalseid ja sensoorset omadusi. Töö uudsus seisneb kommertsiaalsete valgupulbrite funktsionaalsete omaduste süstemaatilises hindamises tingimustes, mis on sarnased toote lõppaplikatsioonile, kombineerituna innovatiivse ja põhjaliku sensoorse analüüsiga, mida on varasemates teadustöödes uuritud vähem. Töö tulemused pakuvad nii teoreetilisi kui praktilisi teadmisi taimsete valkude füüsikalis-keemiliste omaduste ja sensoorsete tajude seostest ning demonstreerivad fermentatsiooni- ja ekstrudeerimistehnoloogia kõrget potentsiaali taimsete valkude kasutusvõimaluste parandamisel jätkusuutlikes ja maitsvates liha- ja piimaalternatiivides.

Appendix 1

Publication I

Jakobson, K., Kaleda, A., Adra, K., Tammik, M.-L., Vaikma, H., Kriščiunaite, T., & Vilu, R. (2023). Techno-functional and sensory characterization of commercial plant protein powders. Foods, 12 (14), 2805. doi.org/10.3390/foods12142805

foods

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Article

Techno-Functional and Sensory Characterization of Commercial Plant Protein Powders

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Abstract: Many new plant proteins are appearing on the market, but their properties are insufficiently characterized. Hence, we collected 24 commercial proteins from pea, oat, fava bean, chickpea, mung bean, potato, canola, soy, and wheat, including different batches, and assessed their techno-functional and sensory properties. Many powders had yellow, red, and brown color tones, but that of fava bean was the lightest. The native pH ranged from 6.0 to 7.7. The water solubility index was 28% on average, but after heat treatment the solubility typically increased. Soy isolate had by far the best water-holding capacity of 6.3 g (H_2O) g^{-1}, and canola had the highest oil-holding capacity of 2.8 g (oil) g^{-1}. The foaming capacity and stability results were highly varied but typical to the raw material. The emulsification properties of all powders were similar. Upon heating, the highest viscosity and storage modulus were found in potato, canola, and mung bean. All powders had raw material flavor, were bitter and astringent, and undissolved particles were perceived in the mouth. Large differences in functionality were found between the batches of one pea powder. In conclusion, we emphasize the need for methodological standardization, but while respecting the conditions found in end applications like meat and dairy analogs.

Keywords: plant proteins; functional properties; water solubility; water-holding capacity; emulsification; foaming; gelling; visco-thermal analysis; sensory analysis

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Citation: Jakobson, K.; Kaleda, A.; Adra, K.; Tammik, M.-L.; Vaikma, H.; Kriščiunaite, T.; Vilu, R. Techno-Functional and Sensory Characterization of Commercial Plant Protein Powders. *Foods* **2023**, *12*, 2805. https://doi.org/10.3390/foods12142805

Academic Editor: Yuliang Cheng

Received: 30 June 2023
Revised: 17 July 2023
Accepted: 21 July 2023
Published: 24 July 2023

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1. Introduction

A growing variety of different plant proteins are used for the development of meat and dairy alternatives, but their properties differ from those of animal proteins and are insufficiently characterized. Furthermore, research and practice show that their properties depend on the plant sources and their variety, growing conditions, processing technology, and purity [1–5]. Extensive mapping of the techno-functional and sensory properties of available plant protein ingredients could certainly support their use.

The important properties for the development of plant-based products include solubility, water- and oil-holding capacities, gelling ability, viscoelasticity, foaming, emulsification, extrudability, cohesion, etc. The nutritional composition and sensory properties like odor, taste, texture, and appearance must be also considered. The relative importance of these properties depends on the product in which they are used. In particular, sausage-type products require ingredients with high gelling and emulsification capacity, while fermented dairy alternatives need high solubility in acidic conditions [6–9].

Based on protein content, ingredients are roughly classified into flours (<50%), concentrates (50–80%), and isolates (≥80%). Usually, dry fractionation using air or wet extraction

with solvents is used to separate proteins from the plant matrices. The dry fractionation technique separates the plant seed components according to their fraction densities. In the wet process, the proteins are first solubilized and then separated using ultrafiltration or precipitation. As a result, protein powders with varying purity and functionality can be obtained [10]. It was shown that using different extraction technologies for pea proteins resulted in protein fractions with varying surface hydrophobicity, gelling capacity, and compressive stress values [11]. In comparison to wet extraction methods, dry fractionation avoids protein denaturation and, thus, tends to preserve the native state and functionality of the proteins [12]. However, denaturation can also be beneficial. Bühler et al. (2020) showed that dry heating of fava bean concentrate reduced its solubility from 53% to 23% but improved its water-holding capacity from 1.3 to 3.1 g g^{-1}, which can enhance juiciness in meat substitutes [13]. The knowledge of protein functionality will help the industry to develop processing methods to target improved properties for specific food applications.

In comparison to lab-scale protein isolation, large-scale commercial production of protein ingredients employs harsher but more efficient and economical processing conditions. Unfortunately, this typically leads to greater protein denaturation and changes in the functional properties in comparison to lab-scale production [14,15]. To date, only a limited number of studies have targeted industrial plant protein powders. Ebert et al. (2020) [5] studied several commercial protein concentrates and isolates, but the investigated properties were limited to protein content, protein solubility at native pH, and appearance. The majority of the samples showed solubility below 20%, although pea samples varied from 8% to 50% [5]. Burger et al. (2022) [14] studied the pH-dependent solubility, emulsification, and droplet surface properties of five commercial pea protein powders before and after homogenization. Four samples had very low crude protein solubility of around 10% at pH 7, but homogenization increased it to 20–55% by reducing the surface hydrophobicity. The solubility was pH-dependent and was the lowest at acidic pH levels of 4–5 (around 5–10%) even after homogenization [14]. Zhao et al. (2020) and Ma, Grossmann, et al. (2022) investigated a broader range of techno-functional properties of commercial plant protein powders, including color, solubility, water- and oil-holding capacities, emulsification, foaming, gelling, and pasting, but with a limited number of samples [16,17]. Ma, Grossmann, et al. (2022) found soy protein isolate to have higher water-holding capacity compared to other pulse proteins like fava bean, pea, and lentil. The oil-holding capacity was similar in soy, fava bean, and lentil but lower in pea. The lentil isolate had the lightest color, while pea was the yellowest [17]. Zhao et al. (2020) [16] correlated protein solubility with the emulsification properties and water absorption capacity. In their study, the functionality of soy was close to that of pea, while rice had lower protein solubility, foaming, and emulsification properties [16].

The market for meat, egg, and dairy analogs is rapidly growing, elevating the demand for protein-rich ingredients [18]. Soy and wheat proteins are the most represented plant proteins on the market, due to their low cost and great functionality. However, these proteins have health, environmental, and nutritional concerns [19–24]. Consequently, pea protein production has emerged, and a wide variety of pea ingredients are already available on the market [25–28]. Many other crops (like fava bean, rice, and oat) are also gaining attention due to their sustainability, hypoallergenicity, functionality, etc. [29–32]. Unlike soy and wheat, which have been in commercial use for decades, these new proteins are less studied, and their functional properties are insufficiently characterized. This hinders the development of new products. Thus, we aimed to comprehensively analyze the sensory and techno-functional properties of a wide selection of commercial plant protein powders, with a focus on potential applications in meat and dairy alternatives.

2. Materials and Methods

2.1. Commercial Protein Powders

We obtained 24 commercial plant protein concentrates and isolates: 9 pea, 4 oat, 4 fava bean, 2 chickpea, and a selection of individual reference samples of mung bean, potato, soy,

wheat, and canola. In addition, the sample set covered different production batches from the same pea and oat products spanning a period of three years. We discuss the samples in more detail in Section 3.1.

2.2. Color Analysis

Samples were placed in 8 cm × 8 cm transparent plastic mini-grip bags, forming a 5 mm thick layer. The color was measured at three different spots in the CIELAB color space using an NS810 Spectrophotometer (3nh Shenzhen Threenh Technology Co., Ltd., Shenzhen, China) set to illuminant D65 and 10°.

2.3. Water Solubility after Heat Treatment at Native pH and pH 4.5

Water solubility was measured gravimetrically at native pH (without adjustment) and at an acidified pH of 4.5 (common for fermented dairy products) in commercial filtered drinking water, mimicking typical industrial processes. Then, 6% powder dispersions were heat treated at 85 °C for 15 min and cooled down in a water bath to room temperature. After that, the native pH was measured using a SevenGo2 pH meter (Mettler Toledo, Greifensee, Switzerland), and the samples were acidified using 10% lactic acid, emulating dairy fermentation. The dispersions were centrifuged for 15 min at 17,290× g, and the supernatants were discarded. The precipitates were washed and centrifuged three times and dried overnight in a thermostat at 105 °C. The acidified samples were washed with acidified water (pH 4.5). Water solubility was calculated from the mass of the dried precipitate relative to the initial powder on a dry weight basis (dwb).

2.4. Water Solubility Index, Water-Holding Capacity, and Oil-Holding Capacity

The methods for measuring oil-holding capacity (OHC), water-holding capacity (WHC), and water solubility index at room temperature (WSI) were adapted from Stojceska et al. (2009) [33]. Briefly, 1 g of powder was suspended in 10 mL of distilled water or rapeseed oil, gently mixed for 30 min at room temperature, and centrifuged at 3000× g for 15 min; the supernatant was carefully decanted, and the remaining precipitate was weighed. WHC and OHC were expressed as the weight of water or oil held by 1 g of powder (dwb). The water supernatant was collected and oven-dried to calculate the WSI, expressed as the percentage of dissolved solids to the initial powder weight (dwb).

2.5. Foam Capacity and Stability

Foams were prepared by dispersing 0.20 g of powder in 20 mL of distilled water, following the protocols of Brishti et al. (2017) and Chandra and Singh (2015), with adaptions [34,35]. The samples were frothed at room temperature inside a 50 mL graduated centrifuge tube using a Polytron PT 2100 homogenizer (Kinematica AG, Malters, Switzerland) equipped with a ∅ 12 mm probe at a speed of 22,000 rpm for 1 min. Foaming capacity (FC) was calculated by measuring the height of the sample volume before (V_1) and after (V_2) frothing and reported as $FC\ [\%] = \frac{V_2 - V_1}{V_1} \times 100$. The change in foam volume (V_t) after 1 h of standing was recorded. Foam stability was reported as $FS\ [\%] = \frac{V_t}{V_0} \times 100$, where V_0 is the initial volume of the foam.

2.6. Emulsion Activity and Stability

Emulsions were prepared by dispersing 0.24 g of powder in 12 mL of distilled water and 12 mL of sunflower oil, following the protocols of Brishti et al. (2017) and Yasumatsu et al. (1972), with adaptations [34,36]. First, samples were mixed at room temperature inside a 50 mL graduated centrifuge tube using a Polytron PT 2100 homogenizer (Kinematica AG, Malters, Switzerland) equipped with a ∅ 12 mm probe at a speed of 11,000 rpm for 1 min. Subsequently, for the determination of emulsion activity (EA), the samples were centrifuged for 5 min at 1100× g at 20 °C. EA was calculated by measuring the height of the emulsified layer (H_1) and the total height of the liquid (H_T) and reported as $EA\ [\%] = \frac{H_1}{H_T} \times 100$. For the determination of emulsion stability after heat treatment (ES),

samples were first heated in a water bath at 80 °C for 30 min, then cooled in an ice-water bath for 15 min, and finally centrifuged for 5 min at 1100× g at 20 °C. For the calculation of ES, the height of the emulsified layer (H_2) was recorded. Emulsion stability was reported as $ES\ [\%] = \frac{H_2}{H_T} \times 100$.

2.7. Rheological Properties

Rheological analyses were based on the methods of Onwulata et al. (2014) and Sun and Arntfield (2010), with modifications [37,38].

The sample dispersions (9%) in distilled water were prepared in 50 mL centrifuge tubes and mixed with a Vortex-Genie Mixer (Scientific Industries Inc., Bohemia, NY, USA) for 1 min. The foam was removed, and 18 mL of the sample was poured into a rheometer's cup.

Gelation behavior was studied with a Physica MCR301 rheometer (Anton Paar, Graz, Austria) equipped with a Peltier C-PTD200 temperature control device and a CC27 concentric-cylinder measuring system. Samples were left to equilibrate at 22 °C for 1 min. Then, the samples were heated over a temperature range of 22–95 °C at a rate of 4 °C min^{-1} while being oscillated at a 1 Hz frequency and 1% strain, which was within the linear viscoelastic region determined in the preliminary tests. A low heating rate is necessary to give protein molecules sufficient time to denature and aggregate [39]. The storage modulus (G′) and loss modulus (G″) were determined as a function of temperature. The samples were run in triplicate, and the maximum value of G′ and the gelling point as a crossover point temperature were reported.

Visco-thermal analysis was performed with a Physica MCR301 rheometer equipped with a C-ETD160/ST electrical temperature control device and a measuring system consisting of a C-CC26/ST aluminum cup and a ST24-2D/2V/2V-30/109 stirrer. To prevent evaporation during the heating cycle, a cover was placed on the top of the cup. The samples were pre-sheared at 25 °C at a rotational speed of 500 min^{-1} for 10 s. Viscosity was measured at a constant rotational speed of 160 min^{-1} throughout five measuring intervals. First, the temperature was kept at 25 °C for 2 min, then it was increased to 85 °C at 12 °C min^{-1}, then kept constant at 85 °C for 5 min, followed by cooling to 25 °C at 12 °C min^{-1} and, finally, was kept again at 25 °C for 2 min.

2.8. Sensory Evaluation

Sensory analysis was conducted at the Center of Food and Fermentation Technologies (TFTAK, Tallinn, Estonia) in a sensory analysis room. The sensory panel consisted of 9 assessors who had previous sensory training and experience with sensory analysis of various plant protein products. All participants from the sensory panel gave consent to take part in the experiment. The participants were informed in advance of the purpose and the procedures of the study. Taking part in the given study was voluntary, and one could withdraw from the test at any time. The participants were in good health and had no known allergies to the components.

The sensory analysis was conducted according to an internal sensory protocol. The samples were prepared as 6% water dispersions using potable water (Saku Läte OÜ, Saku, Estonia). Then, 60 mL of the sample was dosed into the sniffing glass and covered with a lid to avoid the evaporation of the volatile odor compounds. The water dispersions of the samples were mixed vigorously between the pouring to avoid stratification. An additional plant protein sample (Pea 3) was used as a reference in all sensory sessions. All samples were prepared, served, and evaluated at room temperature.

The order of the samples was randomized according to Williams' Latin square design and presented in sequentially monadic order to the panelists. Palate cleansing was carried out between the samples using potable water (Saku Läte OÜ, Estonia), unsalted crackers (Pladis Ltd., London, UK), and slices of pears.

In total, three modalities were assessed: odor, taste, and texture. Odor and taste included attributes such as overall intensity, raw material intensity (e.g., cereals, legumes—depending on the main ingredient), and off-odor intensity. Furthermore, taste also included

bitterness and astringency. The texture modality included "particle size" and "amount of particles". Additionally, the panelists had the option to leave comments on each modality in a voluntary text box.

The assessors evaluated odor and taste attributes on a 10-point scale (0–9), where 0 = "none", 1 = "very weak", 5 = "moderate", and 9 = "very strong". For texture, the scale had different descriptors. For particle size, various points on the scale were described as 0 = "particles missing", 1 = "very small", 5 = "moderate", and 9 = "very big". The scale for the amount of particles, however, was described as 0 = "particles missing", 1 = "few particles", 3 = "some particles", 5 = "several particles", 7 = "many particles", and 9 = "mostly particles". The sensory evaluation results were collected using RedJade software version 3.0.0 (RedJade Sensory Solutions LLC, Martinez, CA, USA).

2.9. Statistical Analysis

All solubility parameters, oil- and water-holding capacities, and gelling analyses were carried out in triplicate. Emulsification, foaming, and visco-thermal analyses were carried out in duplicate. Mean values and standard deviations were calculated. Data visualization and analysis were performed in R 4.3.0 (the R Foundation for Statistical Computing, Vienna, Austria). LOESS (locally estimated scatterplot smoothing) regression was applied to the gelling and visco-thermal results. Spearman's rank correlations and their significance ($\alpha = 0.05$) were calculated with the R package "correlation" 0.8.4 and then visualized with the R package "corrplot" 0.92.

3. Results and Discussion

3.1. Commercial Protein Powders

For our study, we selected products from emerging crops, preferring those that had commercial availability and use. One soy product and one wheat product were selected for comparison. A list of the samples and their protein contents according to the specifications is shown in Table 1. As the functionality of protein powders depends on the crop variety, growing conditions, and processing, we also included different production batches from one pea product and one oat product [4]. In Table 1 and throughout the article, these are marked with asterisks, as well as with green color in figures. The full nutritional and functionality data are available in Supplementary Tables S1–S6.

Table 1. Plant protein powder products and their declared protein contents on a dry weight basis (dwb). Samples marked with asterisks are different production batches of the same product.

Sample	Protein Content, % dwb	Sample	Protein Content, % dwb	Sample	Protein Content, % dwb
Canola	90	Mung bean	85	Pea 5	90
Chickpea 1	89	Oat 1	59	Pea 6 *, 7 *, 8 *, 9 *	80
Chickpea 2	89	Oat 2 *, 3 *, 4 *	56	Potato	90
Fava bean 1	60	Pea 1	85	Soy	90
Fava bean 2	60	Pea 2	85	Wheat	82
Fava bean 3	88	Pea 3	84		
Fava bean 4	60	Pea 4	80		

According to the technical data sheets, 17 out of 24 products were protein isolates with protein contents ranging from 80% to 90%, while 7 samples were concentrates with protein contents of 56–60%. Most pea samples had lower protein content (80–85%) than the soy isolate. This difference was largely due to the presence of fat in pea isolates (5–9%), as commercially important soybean oil is typically extracted before the soy protein isolation process. The lowest protein content of 56–59% was observed in oat concentrates, which in addition to 9–13% fat contents also had a large share of carbohydrates. Three out of four fava bean samples were also concentrates with protein contents of 60%, which is typical for a dry fractionation process [40]. Overall, the average carbohydrate content was 2% in

the isolates and 23% in the concentrates, and the average fat contents were 4% and 7%, respectively. One of the most common parameters provided in the product specification sheets was the pH, which in one case was given as a range of two pH units. Another frequently provided property was the sensory profile, often briefly described as bland, neutral, typical, and characteristic. A few producers stated the country of origin of the plant material, but specific cultivars or varieties were not mentioned. Some specifications included information about the sieve test, color, or descriptive data about the functionality, without including the specific values, methods, and references used in the comparison. Most products, however, had no information about their functional properties, reiterating the need for their characterization.

3.2. Color Analysis

The color measurement results in LAB coordinates are illustrated in Figure 1, where bars show the average color of the raw material. A photograph of the powders is shown in Supplementary Figure S1. Overall, the colors varied considerably between the raw materials, and the pea samples also showed high variance between the products. Lightness values L* (0–100, black–white) were the lowest, at around 74, for potato, canola, and one of the oat samples. The lightest were the fava bean powders, with L* > 90. Lightness was negatively correlated with the green–red a* value (Spearman's $\rho = -0.90$), meaning darker powders were more red, with a* values up to 5, but the range of the blue–yellow b* values was higher in comparison (11–26), indicating the presence of yellow pigments in all of the samples. Significant color variation was noticed between the pea batches; in contrast, the oat batches shared the same color, implying a more stable production process. Our results generally corroborate the findings of Ebert et al. (2020), who reported the lowest lightness for canola and similar color coordinates for commercial pea, potato, and wheat protein powders [5]. However, Ma, Grossmann, et al. (2022) reported much lower lightness values of around 50 for commercial pea, soy, lentil, and fava bean powders [17]. Such a large discrepancy can probably be attributed to the differences in the measurement procedures and the equipment used.

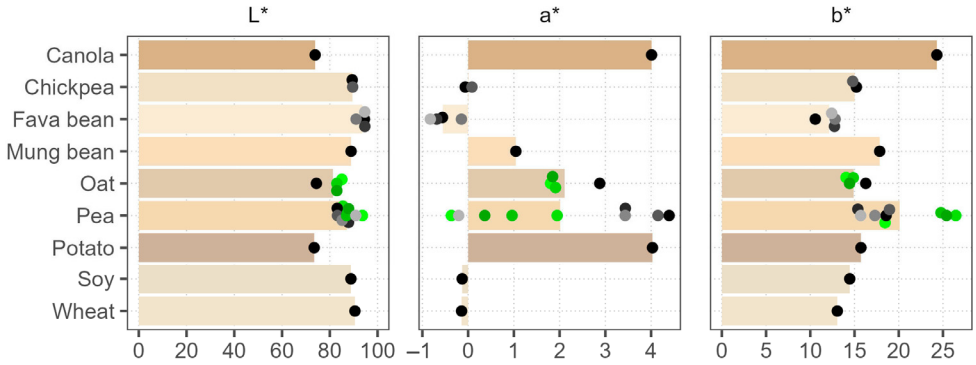

Figure 1. Average powder colors in the CIELAB color space are shown as bar length and bar color. Dots of various shades represent individual products; green color marks different batches of the same product.

Product appearance is the first attribute that consumers assess; therefore, plant protein ingredients should have a light color to facilitate their application in successful product development, especially in dairy replacements [17]. The amount of pigments can vary depending on the plant cultivar and environmental conditions, and they concentrate during the protein isolation process [41,42]. The processing parameters also have a large influence. High temperatures and pH applied during wet production and drying can cause Maillard reactions, caramelization, and oxidation of phenolic compounds and lipids, resulting in a

darker color [43]. Washing, antioxidants, defatting, or milder processing conditions during production could be applied to reduce coloration. Furthermore, smaller particle size was shown to increase the lightness of powders [44]. Thus, the high batch-to-batch variation observed with pea products could reflect changes in any of the listed factors.

3.3. pH and Solubility

3.3.1. Native pH

The solubility of proteins strongly depends on pH and increases as the pH deviates from the isoelectric point, which for plant proteins is often around pH 4–5 [4]. A notable exception is wheat gluten, which has an isoelectric point around pH 7 [16]. The native pH of our samples in 6% water dispersions after heat treatment was in the range of 6.0–7.7. The highest pH was found in soy. Oat, canola, fava bean, and wheat were mildly acidic at pH 6.0–6.7. Most of the pea samples had pH 7.1–7.6, and large differences between the pea batches were observed, where the pH varied from 6.6 to 7.1. All of the oat batches were consistently at pH 6.6. Ebert et al. (2020) measured the native pH of 3% dispersions without heating and observed even wider pH ranges in commercial protein isolates: pea 5.8–7.7, wheat 5.4–6.1, canola 8.1, and potato 3.3–8.1 [5]. In the dry fractionation process, chemicals are not used, and the pH of the protein concentrate corresponds to the initial pH of the raw material, but in the wet fractionation process based on protein solubilization and precipitation via pH changes, the final pH is chosen by the producer [12]. Because solubility and other functional properties like gelation, emulsification, or extrudability strongly depend on pH, it is important to match the pH of the protein isolate to the final product application, as additional pH adjustments are undesirable [16,45,46]. Furthermore, researchers investigating the functionality of protein isolates in their native state should always report the pH, as it can significantly differ even between batches of the same commercial product. Unfortunately, in many relevant studies, the native pH was not reported [14,16,17,46].

3.3.2. Solubility

We measured powder solubility using three different methods. The water solubility index (WSI) assesses solubility at room temperature and native pH, and this is often used in research, but the other methods apply heat treatment and investigate solubility at two pH points: native and pH 4.5. Moreover, the distinction between whole-powder solubility and protein solubility is important, as protein concentrates (unlike isolates) also contain a significant portion of carbohydrates. In a liquid product, any undissolved particles of the whole powder may sediment, causing sensory defects. Thus, the second and third methods are more relevant to the industry, as products are pasteurized to guarantee microbiological stability, and pH 4.5 is a typical value for fermented dairy analogs.

The WSI results are shown in Figure 2 as dots in various shades of gray and black. Canola and potato protein isolates were almost completely soluble at room temperature, and these products are marketed as such. But in general, the WSI of the powders was below 60%, and on average it was 28%. The lowest WSI (in the range of 4–10%) was observed in wheat, oat, six out of nine pea samples, and one fava bean isolate. The other fava bean samples, which were protein concentrates, had considerably higher WSI of 40–60%. Batch-to-batch WSI variation in pea products was very high (fourfold), but the oat batches were consistent.

The powder solubility after heat treatment is shown in Figure 2 as lines, facilitating the comparison of two pH levels. Solubility at native pH after heat treatment was generally higher than WSI measured at room temperature, but the increase in solubility was greatly dependent on the product. Pea samples were the ones that derived the greatest benefit. The heat treatment step improved the solubility of pea samples at native pH by up to 36%. Pea 5 and soy became 93% soluble, making them especially suitable for liquid applications at neutral pH. Oat, wheat, and chickpea showed only modest improvements of up to 10%, and their solubility remained low. On the other hand, fava bean and mung bean either

showed little improvement or even became less soluble. Canola and potato were special cases because heat treatment caused gelation; therefore, their low solubility results instead show that the gels retained the entrapped solutes during the measurement procedure.

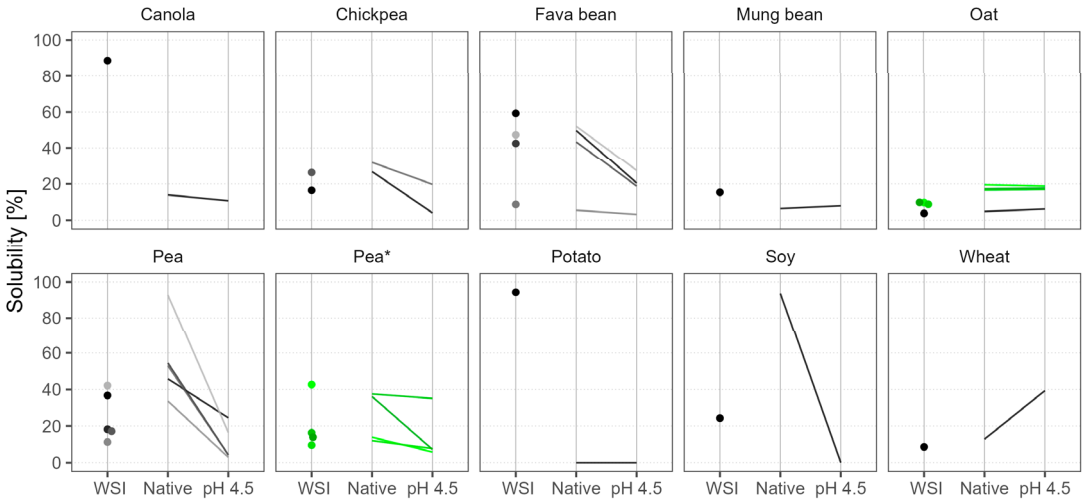

Figure 2. Water solubility of commercial plant protein powders according to three different methods. The water solubility index (WSI) was measured at room temperature and native pH. The other methods involved heat treatment and two pH points: native pH and pH 4.5. Dots and lines of various shades represent different products; green color and the asterisk mark different batches of the same product.

As expected, solubility at acidic pH (4.5) after heat treatment was considerably lower in most of the samples. On average, the decrease was threefold, but the sharpest drop was seen in Pea 5 and soy samples, which at their native pH were almost completely soluble. The acidification had little further effect on powders that were poorly soluble at native pH, like oat, mung bean, and some pea and fava bean samples. The only exception was wheat, which became more soluble when the pH shifted away from its isoelectric point at around pH 7.

The differences in powder solubility after heat treatment between the oat batches were insignificant, but the changes between the pea batches were surprising. At native pH, two batches were 13% soluble and two were 37% soluble, but at pH 4.5 the solubility dropped to 7%, except for Pea 8*, which maintained its good solubility and even became the most soluble pea sample in acidic conditions. These large and inconsistent variations between batches pose a challenge for the food industry, as a product developed with one batch might not be possible to replicate with another.

Other researchers have also noted the problematic solubility of commercial plant protein powders, although they have studied crude protein solubility without the heat treatment step. Ebert et al. (2020) found that pea, wheat, rice, sunflower, and pumpkin protein isolates and concentrates had low solubility at pH 7, in the range of 4–50%, unless they were specifically developed to be highly soluble like some potato and canola products [5]. In a study by Burger et al. (2022), five pea samples had only 10% protein solubility at pH 7, but after homogenization this increased to a range of 19–56% [14]. Conversely, at pH 5, the solubility stayed low despite the homogenization process. This confirms that the solubility of plant protein ingredients remains a challenge and requires additional steps like homogenization and heat treatment to improve their functionality. Moreover, some producers of plant protein ingredients recommend these technological steps in their application guidelines.

3.4. Water- and Oil-Holding Capacities

Water-holding capacity (WHC) and oil-holding capacity (OHC) describe the ability to hold water or oil during the application of forces [47]. Factors that influence these properties include protein size, amino acid composition, conformation, and carbohydrate and fat contents. Protein powders with more hydrophilic groups have higher WHC due to the formation of a higher number of hydrogen bonds. Likewise, OHC can be explained by hydrophobic and nonpolar side chains on the surface of particles that could interact with oil molecules [48].

We illustrate the WHC and OHC results in Figure 3. Soy had by far the highest WHC of 6.3 g (H_2O) g^{-1}, followed by Pea 5, with a WHC of 3.5 g (H_2O) g^{-1}; both were also notable for their high solubility at native pH. The unmatched WHC of soy in comparison to pea, rice, and wheat was also reported by Zhao et al. (2020) [16]. Pea samples, including different batches of the same product, were highly varied, with WHC values in the range of 0.9–3.5 g (H_2O) g^{-1}. Chickpea, fava bean, mung bean, oat, and wheat tended to have WHC below 2.6 g (H_2O) g^{-1}. Our WHC measurement methodology discards the dissolved part of the sample and considers only the water held by the undissolved part. Consequently, WHC was negatively correlated with WSI among samples with values below 1.3 g (H_2O) g^{-1} and above 25% WSI. After the exclusion of these samples, the lowest WHC was found in oat and wheat, at 1.5 g (H_2O) g^{-1}. For the same reason, the WHC of canola and potato was not measured, as they dissolved almost completely. In comparison to WHC, OHC was generally lower and with less variation between the samples. The highest OHC was seen in canola and potato, at 2.8 and 2.1 g (oil) g^{-1}, respectively. All of the other samples varied between 0.8 and 1.7 g (oil) g^{-1}, and the batch-to-batch variation of both pea and oat products was small. Similar to our findings, Fuhrmeister and Meuser (2003) reported the highest WHC in a commercial soy protein isolate, followed by a pea protein isolate, at 4.6 and 4.0 g (H_2O) g^{-1}, respectively [49], but the OHC of their soy (1.2 g (oil) g^{-1}) was lower in comparison to our soy sample (1.6 g (oil) g^{-1}), and also compared to their commercial pea protein isolate (1.6 g (oil) g^{-1}).

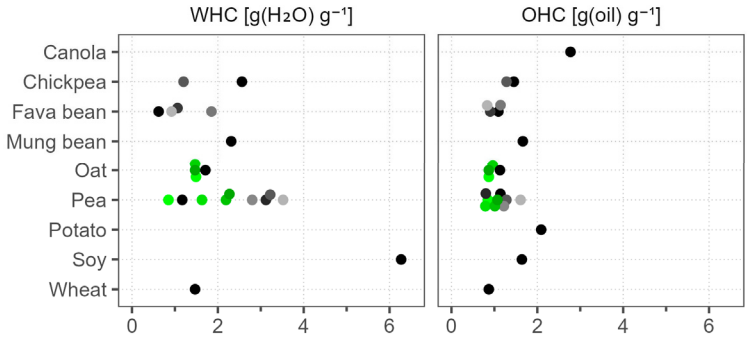

Figure 3. Water-holding capacity (WHC) and oil-holding capacity (OHC) of commercial plant protein powders. Dots of various shades represent different products; green color marks different batches of the same product.

The juiciness of plant-based extruded meat analogs was shown to depend on the WHC of the raw material. Furthermore, the generally recommended WHC value for meat analogs is above 3 g (H_2O) g^{-1} [50]. In our study, only soy and three pea samples were above this threshold, with soy demonstrating a remarkably higher value compared to pea. Our results reveal that in this specific application soy proves to be challenging to replace with alternative protein sources.

3.5. Foaming and Emulsification Properties

The foaming and emulsification properties of plant-based ingredients are important for products such as dressings and sauces, as well as alternatives to ice cream, whipped cream, egg, and milk. Adsorption to air–water or oil–water interfaces facilitates the formation and stabilization of gas bubbles or oil droplets by creating a protective coating that generates repulsive forces and introduces some mechanical rigidity that inhibits aggregation [8].

We present the foaming capacity (FC) and foaming stability (FS) results in Figure 4. The FC results were highly variable, covering the whole scale, yet specific raw materials had a characteristic range of values. The highest FC was in wheat (98%) and potato (95%), followed by chickpea and canola, but the lowest was in oat samples (9–19%). The batch-to-batch variation was small. Other studies corroborate the excellent FC of wheat, and that soy has a higher FC compared to pea and fava bean [7,51]. Some reports have attributed high FC to high protein solubility, but our wheat and oat samples both had similarly poor solubility, yet the opposite FC results [52]. Therefore, other factors in addition to solubility are probably more important for foaming, like molecular flexibility and hydrophobicity [53]. The FS results, however, were notably different from the FC results. Foams made with potato and wheat powders were less stable in comparison to soy, which had twofold lower FC. Instead, mung bean, with a low FC of 25%, produced the most stable foam, confirming the results of Tang et al. (2021), who found that mung bean had superior FS compared to soy, chickpea, lentil, pigeon pea, and cowpea [54]. Oat samples, in addition to having the lowest FC, also had FS close to 0%. The FS of pea samples, including different batches, varied in a wide range of 31–68%, but because their FC was relatively low, a higher variance in foam stability measurements was anticipated.

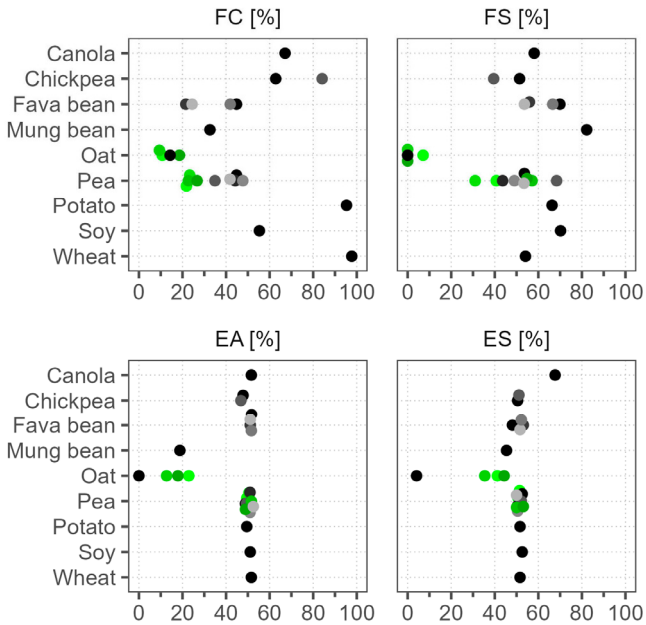

Figure 4. Emulsification and foaming properties of commercial plant protein powders. Dots of various shades represent different products; green color marks different batches of the same product. FC—foaming capacity, FS—foaming stability, EA—emulsification activity, ES—emulsification stability.

The emulsification activity (EA) and emulsification stability (ES) results are shown in Figure 4. The EA results were mostly around 50%, but oat and mung bean were below 23%, indicating poor emulsification functionality. To confirm these results, we increased the

powder concentration during emulsification by 3.5-fold, yet the results were still around 50%. In a study by Tang et al. (2021), a commercial soy protein isolate also had an EA of 54%, much lower than that of their lab-produced isolate with an EA of 71% [54]. Poor emulsification properties of oat and mung bean have also been reported and associated with low solubility [54,55]. Peng et al. (2016) found that pea proteins formed more stable emulsions after heating [56]. In our ES measurement procedure, a heat treatment step was applied, and it generally improved the solubility, but not in mung bean or Oat 1 (Figure 2). We found that heat treatment increased the ES of mung bean to 45% and that of the other oat batches to 35–44% but had no effect on Oat 1. The most notable sample was canola, where the ES improved to 68%. This could be related to its gelling behavior after heating, yet the potato sample that also gelled showed no increase in ES [53]. The ES results of most other samples were in the range of 48–53%, unchanged from the EA. This also confirms that solubility is not the only factor that determines emulsification functionality. The batch-to-batch variation of the EA and ES results between the pea samples was small. Although the variance was higher between the oat batches, this can be disregarded because of their low emulsification functionality.

3.6. Visco-Thermal Analysis

A visco-thermal profile reflects the viscosity under a designated heating and cooling regime during constant mixing. This analysis is typically used for assessing the functional properties of starch, which is mainly found in flours and dry-fractionated protein concentrates. In the case of protein isolates, it could show the changes in viscosity due to protein solubilization, denaturation, and gelation after heat treatment and physical processing [37]. In some applications, such as plant-based milk alternatives, the increase in viscosity is considered objectionable. However, in plant-based yogurts and desserts, the viscosity increase is appreciated, and in meat analogs the gelling effect is required [57].

We present the visco-thermal profiles in Figure 5, with the temperature regime outlined by a blue line for guidance. The soy sample had the highest initial (0 min) viscosity of 501 mPa s, followed by a few pea and chickpea samples with viscosities in the range of 119–210 mPa s. For reference, an ordinary dairy yogurt could have a viscosity of around 300 mPa s [58]. The other samples had more modest viscosity values (10–90 mPa s). After heating to 85 °C, at the 12 min point, many samples showed considerable changes in viscosity. The potato sample had the largest viscosity increase, from 13 to 3716 mPa s, which could be related to the gelling behavior that was observed during the solubility analysis. The hot viscosity also increased in mung bean and canola to around 200 mPa s. In contrast, the hot viscosity of some pea samples and soy significantly dropped—in the case of soy, by 24-fold to 21 mPa s. These were the same samples that became considerably more soluble after heat treatment. In the end (19 min), after the cooling step, the viscosity changes in these samples were small. However, mung bean and a few chickpea, fava bean, and pea samples showed an additional increase; thus, their end viscosity greatly exceeded their initial viscosity. The most notable was the potato protein isolate, which after the cooling step achieved the highest overall viscosity of 8857 mPa s. All oat samples, wheat, and some pea, chickpea, and fava bean samples had relatively small viscosity values, making them suitable for applications where high viscosities are not desired. Variation between the oat batches was minimal; in contrast, the pea batches diverged in their behavior during the measurement sequence.

Other studies have used different measurement parameters like powder concentration, temperature, and time, making it difficult to directly compare the results. Osen et al. (2014) measured the visco-thermal profiles of three commercial pea protein powders using different powder concentrations for each of them (15–20%) and a temperature profile of 50–95–50 °C [46]. Two of their pea samples showed a high initial viscosity of around 2000 mPa s, which at 95 °C decreased to 200–350 mPa s, but the third sample had an initial viscosity of around 70 mPa s, which at 95 °C instead increased to 530 mPa s, making the viscosity of all three samples more similar in the end. They related the high initial viscosity

to low solubility and high WHC and explained that, upon hydration, the proteins absorb water and swell, but when soluble proteins with low viscosity denature during heating their solubility decreases and, thus, their viscosity increases. Nevertheless, the observations of our results suggested more intricate trends. While the initial viscosity and WHC were indeed strongly correlated (Spearman's $\rho = 0.89$), no significant correlation was found between viscosity and WSI. Supporting our results, Webb et al. (2023) demonstrated considerable differences in the visco-thermal profiles between commercial pea products, which could stem from their different cultivars, growing environments, and protein isolation methods [59]. Furthermore, a mixture containing a higher starch content and lower protein content was shown to provide higher end viscosity compared to a mixture containing the highest protein content [60,61]. Conversely, our results showed much lower end viscosity in the starch-containing fava bean concentrates than in the fava bean sample with the highest protein content.

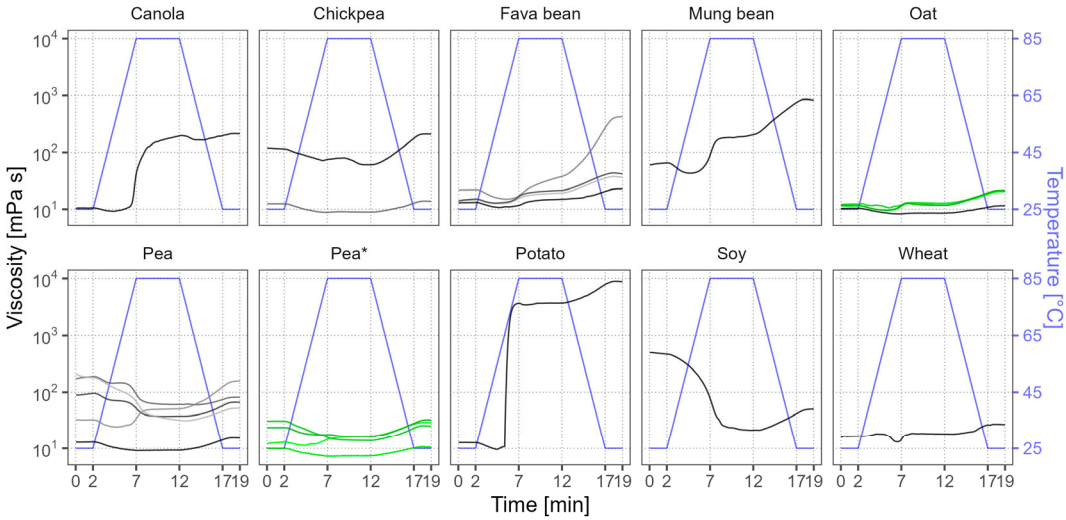

Figure 5. Average visco-thermal profiles of commercial plant protein powders. Lines of various shades represent the viscosity profiles of different samples, and green color and the asterisk mark different batches of the same product. The blue line shows the temperature profile during measurement.

3.7. Gelation Behavior

The rheological behavior during heating reveals the thermally induced gelling functionality of powder dispersions in water. Gel formation is important for foods like meat, fish, and egg analogs. Unlike the visco-thermal analysis, during gelation, the sample was maintained in a quiescent state without continuous stirring. The storage modulus G′ represents the solid-like behavior and the loss modulus G″ represents the liquid-like behavior of the sample. When G′ is higher than G″, the sample matrix exhibits gel-like properties, but in this study we consider the structures with G′ < 10 Pa as too weak and, hence, irrelevant in practical applications. For reference, an ordinary dairy yogurt could have G′ on a scale of 100 Pa [62,63].

The majority of our samples had G′ > G″ at 95 °C (Figure 6), indicating more solid-like behavior and the presence of a three-dimensional macromolecular network within the sample. Only the three oat batches and Pea 7* were notable for their more predominant viscous behavior (G′ << G″). The differences between the oat batches were minor, while the pea batches showed more variance. However, overall, almost all of the gels were too weak, as the absolute G′ values were < 10 Pa, except for mung bean, potato, soy, and canola. The G′ value of mung bean gradually increased during heating above 28 °C and reached 1720 Pa, but in potato the increase occurred rapidly between 65 °C and 80 °C, and

the highest G′ was 865 Pa—twofold lower than that of mung bean. When these samples were cooled and removed from the rheometer cell, the texture of the gels was firm and, in the case of potato, marmalade-like. Soy performed differently and formed a gel-like texture already at room temperature with G′ = 296 Pa, but during heating the texture became more liquid as both G′ and G″ dropped to 2 Pa. This behavior was similar to the observed viscosity trend in the first half of the visco-thermal analysis. Canola also showed some gelling functionality, but its gel was weak (G′ = 15 Pa), supporting the findings of Yang et al. (2014), who reported similar G′ values at neutral pH and demonstrated that higher pH was needed for stronger thermal gelation of canola [11]. Gelling ability is also stronger at higher protein concentrations in the solution, as it increases the chance of intermolecular interactions, and there is a minimum concentration of protein below which a continuous three-dimensional structure cannot be formed [53]. This could explain the low G′ results of the oat batches, as their protein contents and solubility were also low. The differences in the gelling ability of the same crop could be attributed to the crop variety, protein extraction method, and other components in the powder [64]. Starch and fiber contents can influence the final textural properties of the gel, mainly reducing gel fracture stress and strain, making the gel weaker and more brittle [65]. Hydrocolloids like oat β-glucans, on the other hand, can have synergistic effects with proteins, increasing the gel strength [66,67].

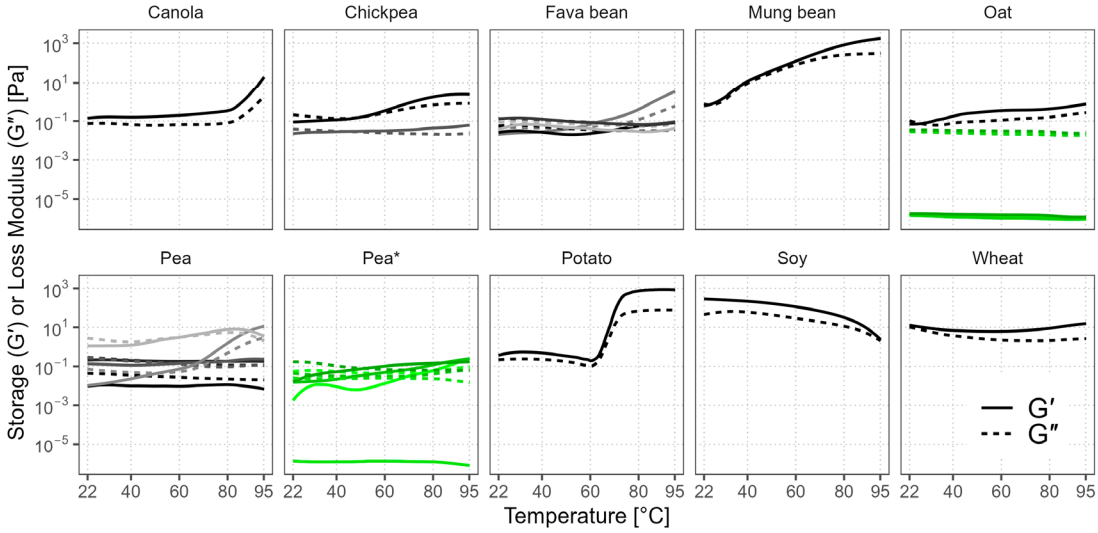

Figure 6. Storage modulus (G′) and loss modulus (G″) of commercial plant protein powders during heating are shown as solid and dashed lines of various shades. Green color and the asterisk mark different batches of the same product.

3.8. Sensory Evaluation

Unfamiliar and undesirable sensory properties cause lower consumer acceptability of plant-based alternatives [68,69]. Therefore, the development of successful plant-based meat and dairy alternatives requires ingredients with no specific odor or taste. Then, flavor compounds can be added to achieve the desired flavor profile of the final product. However, research shows that plant-based end-products tend to have different off-odors and off-tastes derived from protein powders [70].

As shown in Figure 7, almost all of the evaluated products had an intense overall and raw material odor, with scores in the range of 5.3–8.7. Only chickpea had no specific raw material odor, yet its overall odor was intense. The overall taste intensity and raw material taste attributes were more varied but also quite high (3.4–8.0). In general, chickpea, fava

bean, mung bean, and canola had the most intense odor and taste profiles, while soy, oat, wheat, and some pea samples were the least intense. The variation in these taste and odor attributes was evident within the same raw material, e.g., the range of variation between the pea samples was up to 3.2 units. Off-odors and off-tastes were not very apparent (scores < 2.2), since the overall intensity was mostly dominated by the raw material. The exceptions with higher off-flavors were mung bean (rubbery and dusty off-odor, soapy and moldy off-taste) and Pea 9* (sulfurous off-odor).

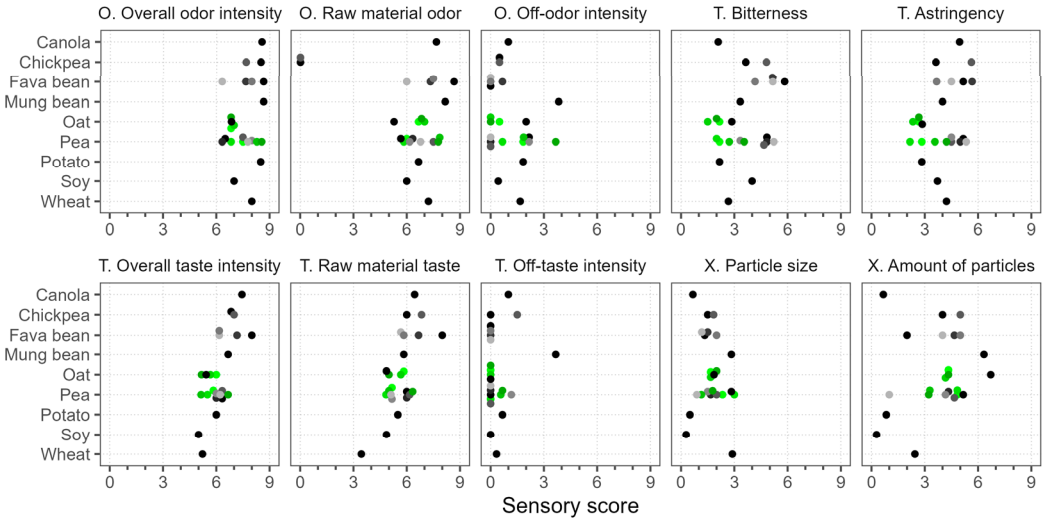

Figure 7. Sensory analysis of commercial plant protein powders. Scale (0–9) is defined as "none"–"very strong" for sensory attributes, "particles missing"–"very big" for particle size, and "particles missing"–"mostly particles" for particle amount. Dots of various shades represent different products; green color marks different batches of the same product. O.—odor, T.—taste, X.—texture.

The samples were rather different in terms of bitterness and astringency, with scores ranging from 1.5 to 5.8, and some characteristics were dependent on the raw material. For example, fava bean samples tended to be generally more bitter and astringent, whereas oat was generally evaluated lower in terms of these attributes. However, bitter compounds vary depending on the plant protein source, and their perception depends on the physiological differences, even among the trained panelists [71]. Thus, consumers' perceptions of different raw materials may also vary.

The low solubility of plant protein powders was also problematic, because the assessors detected particles in most of the samples (Figure 7). The largest particles, with scores of around 3.0, were found in wheat, mung bean, Pea 1, and Pea 7*. The particle size of the other pea samples varied between 0.9 and 3.0. The samples were more distinguished by the "amount of particles" attribute. The highest scores were given to Oat 1 and mung bean, at 6.7 and 6.3, respectively, which based on the scale translates to between "several" and "many" particles. The "particle size" and the "amount of particles" attributes were correlated (Spearman's $\rho = 0.74$); thus, the lowest scores in both attributes were found in soy, canola, potato, and Pea 5 (scores ≤ 1.0). These samples were also the most soluble.

The sensory differences between the oat batches were small, but the pea batches varied greatly, especially in their odor profiles. Two of the pea batches were more intense in odor and also had more off-notes, described as sulfuric and cheesy, while the other two had green, sweet, and rhubarb-like nuances. These characteristics may have influenced the overall sensory profile and, hence, the perception of other attributes like astringency and bitterness, which were also graded higher in these samples. This indicates that the sensory

properties of plant protein powders can be influenced by the production method, plant cultivar, and growing conditions [41,70].

3.9. Correlations

Figure 8 shows statistically significant Spearman's rank correlation coefficients, which were multiplied by 100 for brevity. The protein content in the powder had the strongest positive correlations with the WHC (ρ = 0.78), FC (ρ = 0.78), and OHC (ρ = 0.69), and less with the rheological parameters (ρ = 0.45–0.62). This indicates that these functional properties could be attributed more to proteins than carbohydrates, contradicting studies that showed higher viscosity with lower protein and higher starch contents [60,61]. On the other hand, the protein content was not correlated with the three solubility measures, nor with the sensory attributes. This implies that for liquid end-product applications, assessment of just protein solubility instead of whole-powder solubility is insufficient to predict the functional properties of a protein powder, as other constituents could also play a major role.

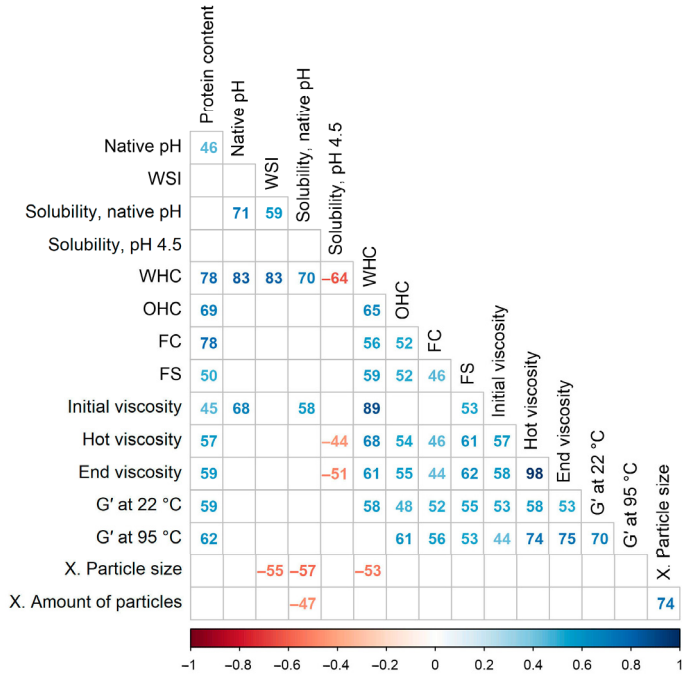

Figure 8. Spearman's rank correlations between the techno-functional properties of commercial plant protein powders. Multiplied by 100 for brevity. Only statistically significant results are shown (α = 0.05). WSI—water solubility index, WHC—water-holding capacity, OHC—oil-holding capacity, FC—foaming capacity, FS—foaming stability, G′—storage modulus, X.—sensory texture.

The native pH and solubility at native pH after heat treatment were positively correlated (ρ = 0.71). Therefore, in the end-products where the pH is not changed, the selection of a protein powder with a higher pH could be more beneficial, as it will be more soluble. Because the native pH of a protein isolate is chosen by the producer, this enables tailoring of the isolation process to specific applications. However, the native pH was not correlated with the WSI, which is measured at native pH and room temperature. Furthermore, the three different solubility parameters were not strongly intercorrelated ($\rho \leq 0.59$). This means that to facilitate powder selection, a suitable solubility measurement method must be chosen according to the product being developed, as high cold solubility at native pH

does not guarantee that the powder will remain dispersed after product fermentation and pasteurization.

After the exclusion of samples with low WHC and high WSI, as described in Section 3.4, the WHC was found to be strongly correlated with the protein content (ρ = 0.78), native pH (ρ = 0.83), WSI (ρ = 0.83), and solubility at native pH (ρ = 0.70). These findings are in line with the results of Zhao et al. (2020), who studied commercial pea, soy, rice, and wheat protein isolates and concentrates and found a significant correlation between protein solubility and water absorption capacity (Pearson's r = 0.99) [16]. Regarding the correlation between WHC and protein content, it could be partially explained by the decrease in fat content, as defatting was shown to increase the WHC of flours [72]. The capacity of proteins to bind and retain moisture depends on the type and number of polar hydrophilic groups that are available to bind water. The pH value influences the conformational state of proteins and protein–protein interactions, which, in turn, could hide or reveal those water-binding groups [53]. Surprisingly, the correlation between WHC and solubility at pH 4.5 was negative (ρ = −0.64), probably because samples with a larger number of available polar groups and good water binding aggregated more tightly under acidic conditions and, thus, were less soluble instead. Furthermore, WHC was correlated with the initial viscosity (ρ = 0.89), corroborating the results of Osen et al. (2014), who reported that protein swelling was responsible for the increase in viscosity [73]. However, in our study, the correlations between the WHC and viscosity after the heating and cooling steps decreased to 0.68–0.61. These values are similar to the correlation between water absorption capacity and peak viscosity during heating (Pearson's r = 0.7) reported by Webb et al. (2023) for commercial pea, soy, and wheat protein powders [59].

The OHC was positively correlated with the protein content and WHC (ρ = 0.69–0.65). Because proteins have hydrophilic and hydrophobic groups, the increase in protein content could have increased the amounts of both groups and enhanced the water and oil binding simultaneously.

The FC was most strongly correlated with the protein content (ρ = 0.78), but not with the three solubility parameters. Potentially, other powder components like fibers could have interfered with the adsorption of proteins to the air–water interface. The FS was most correlated with the WHC and viscosity of the solution; in other words, the FS was higher at lower water mobility conditions. The foaming functionality depends on pH and is the highest at the isoelectric point, where aggregated proteins form thick interfacial films, but in our work the correlation between the native pH and the foaming properties was not observed [53].

Among the visco-thermal parameters, the strongest correlation was found between the hot and end viscosities (ρ = 0.98); hence, their correlations with the other parameters were also similar. However, the initial viscosity before the heating step was different; it was correlated with the WHC and native pH—parameters related to protein swelling. After the heating and cooling steps, however, these correlations decreased. Instead, the hot and end viscosities were most strongly correlated with the storage modulus at 95 °C (ρ = 0.74–0.75); in contrast, the correlation between the storage modulus at room temperature and initial viscosity was low.

Finally, the solubility parameters were negatively correlated with the sensory characteristics of textural granularity. This confirms that plant protein powders must be soluble to a considerable extent for their successful application in dairy alternative products; otherwise, the undissolved particles would be perceived in the mouth, causing sensory defects.

3.10. General Discussion

Our results demonstrate that the properties of commercial plant protein isolates and concentrates vary considerably, even within the crop groups. Hence, for successful end-product development, specific protein powders must be selected according to the required functionality. Unfortunately, manufacturers rarely provide this information, making the selection process more challenging.

Typically, studies of protein functionality are performed with protein isolates produced in laboratory facilities employing milder temperature regimes compared to those used in the production of commercial products. Consequently, the properties of laboratory-produced isolates may differ from their commercial counterparts. The current surveys of commercial protein powders include either a small selection of powders or a limited list of investigated techno-functional properties. In addition, various studies employ different measurement procedures and analysis conditions, making comparison of the results more difficult. This implies that methods should be standardized, and the selection of the analysis conditions must be directed by the end applications and consider possible changes that may occur with the proteins during further processing.

We should highlight the lack of strong correlations between the three different solubility measures that we investigated. Heat treatment mostly increased the solubility but also made the method unsuitable for the cases where it caused gelation. The solubility of the protein ingredients generally decreased at pH 4.5, which is particularly relevant to fermented products, but these changes were highly inconsistent between the protein powders, reiterating the need to select the solubility metric according to the end application. In addition, while studies commonly measure the solubility of protein only, the dispersibility of the whole powder cannot be overlooked, as other components like carbohydrates and undissolved particles influence the techno-functional and sensory properties. This is particularly crucial for less-refined protein concentrates.

The color of protein ingredients remains a significant challenge in many applications. Many samples were dark and had red, yellow, or brown tones, which especially limits their use in dairy alternatives but also creates difficulties in achieving meat-like colors during the development of meat analogs. Even though the measurement of color is a straightforward procedure, comparison with the literature can still present challenges. For example, the lightness values reported by Ma, Grossmann, et al. (2022) for commercial powders were 20 units lower than our darkest samples, which were already visually perceived as very dark [17]. Furthermore, Tang et al. (2021) noted that lab-scale powders could be lighter compared to commercial ones due to a milder drying process (lyophilization vs. spray-drying) [54].

All samples exhibited clear raw material flavors, along with bitter and astringent tastes. However, some protein ingredients were significantly more favorable than others, and soy generally emerged as one of the best performers. Different approaches have been proposed to improve the flavor of plant proteins, including cultivar selection and breeding; pretreatments with germination, heating, enzymes, or fermentation; optimization of extraction methods, end-product processing, and storage; taste-masking techniques, and others [74]. Unfortunately, additional processing steps can increase the cost of already-expensive ingredients, particularly when compared to soy.

While the different batches of the same oat sample were consistent, almost all techno-functional properties of the pea batches varied considerably, including native pH, solubility, viscosity, and sensory parameters. Indeed, this poses a challenge for a food production process that has been optimized for a specific batch of plant protein. Therefore, it is essential to monitor the inter-batch functional performance of the plant protein ingredients. WHC, a rather simple analysis, was strongly correlated with many other functional parameters. Thus, WHC, in addition to the native pH and WSI, which can be measured together with WHC, are the simplest and the most accessible methods for monitoring functional performance. Ideally, these parameters could be provided by the manufacturers on their specification sheets as typical functionality indicators of the plant protein ingredients, giving valuable information to their customers.

For future research, we recommend including a wider range of crops and a larger number of representative samples produced using different technologies. To facilitate the comparison between different studies, the characterization methods and their conditions should be standardized, including factors like powder or protein concentration, pH, temperature, and time. We found that native pH has a significant influence on the functional

properties of protein powders; thus, future studies should always report it. Furthermore, in the sensory analysis, we focused on a few generalized attributes, but the actual sensory profile of the products was more diverse; therefore, future works could investigate the sensory characteristics in more detail. In addition, our study demonstrated that the acidification and heating steps have a substantial impact on the properties of protein powders, so these treatments could also be incorporated into the sensory and functionality assessment methods.

To conclude, we performed a comprehensive mapping of the techno-functional and sensory properties of commercial plant protein isolates and concentrates. Soy protein isolate demonstrated the best overall properties in our study. However, when considering specific functionality, alternatives to soy protein isolate were also identified. Canola protein isolate had better oil absorption capacity, potato protein formed strong gels, and some pea and fava bean samples were more soluble under acidic conditions (relevant for fermented dairy analogs). Our results highlighted large variations in the properties between different crops, within the crop groups, and between the batches of the same product. Finally, we should emphasize the need for standardized techno-functional characterization methods to support the development of meat and dairy alternatives and to ensure the uniform quality and stability of these products. This, in turn, would contribute to increased adoption and acceptance of novel sustainable food options.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/foods12142805/s1, Figure S1: Photo of commercial protein powders prepared for color measurement; Table S1: Nutritional composition according to the manufacturers' specifications and native pH; Table S2: CIELAB color. Mean (SD, n = 3); Table S3: Solubility. Mean (SD, n = 3); Table S4: Water- and oil-holding capacities. Mean (SD, n = 3); Table S5: Foaming and emulsification properties. Mean (SD, n = 2); Table S6: Sensory properties. Mean (SD, n = 9).

Author Contributions: Conceptualization, K.J., T.K. and A.K.; methodology, K.J., T.K. and A.K.; software, A.K.; validation, K.J., A.K., T.K., H.V. and M.-L.T.; formal analysis, K.J., A.K., K.A., H.V. and M.-L.T.; investigation, K.J., K.A., H.V. and M.-L.T.; resources, T.K.; data curation, K.J., A.K., T.K., K.A., H.V. and M.-L.T.; writing—original draft preparation, K.J., K.A., H.V. and M.-L.T.; writing—review and editing, K.J., A.K., T.K. and R.V.; visualization, A.K.; supervision, T.K. and K.J.; project administration, T.K.; funding acquisition, T.K. All authors have read and agreed to the published version of the manuscript.

Funding: The financial support for this research was provided by the European Regional Development Fund (ERDF) and the Estonian Research Council via project RESTA16.

Data Availability Statement: The data are contained within the article and its Supplementary Materials.

Acknowledgments: The authors wish to thank Viktoria Soboleva, Kristiina Loit, Katarin Mägi, and Kaisa Valge for their contributions to the experimental work.

Conflicts of Interest: The authors declare no conflict of interest.

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Appendix 2

Publication II

Jakobson, K., Kaleda, A., Vaikma, H., Sats, A., Rosenvald, S., & Kriščiunaite, T. (2026). Particle size and surface morphology of plant protein powders determine the sensory perception of graininess in liquid matrices. Applied Food Research, 6(1), 101699. doi.org/10.1016/j.afres.2026.101699

Applied Food Research 6 (2026) 101699

Contents lists available at ScienceDirect

Applied Food Research

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

Particle size and surface morphology of plant protein powders determine the sensory perception of grittiness in liquid matrices

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ARTICLE INFO

Keywords:
Plant proteins
Particle surface morphology
Particle size analysis
Sensory analysis
Scanning electron microscopy
Visual sensory assessment

ABSTRACT

Plant protein powders are used in liquid dairy alternatives, but their low solubility may cause gritty texture defects. We investigated the factors influencing the sensory perception of particle size in 18 commercial plant protein powders—chickpea, fava bean, mung bean, oat, pea, soy, and wheat—in heat-treated water dispersions at native pH or pH 4.5, conditions relevant to end applications. We measured particle size distribution using laser diffraction and compared it with a sensory assessment. We also used scanning electron microscopy to visualize particles and developed a unique method to quantify surface morphology attributes by visually grading images with a sensory panel, obtaining particle angularity, surface roughness, and heterogeneity characteristics. The results showed that sample preparation significantly influenced particle size distribution and there was no correlation between instrumental results in the dry and liquid state. Particle sizes (D90) in water dispersions at native pH ranged 75–375 μm, which increased to 73–493 μm at pH 4.5; soy and wheat particles formed large clumps of 1–2 mm. We observed a linear relationship between D90 and sensorially perceived particle size for particles over 200 μm. For smaller particles, the perceived sensory size was instead explained by particle angularity and surface roughness. Small, round, and smooth particles were imperceptible, while rough and angular particles of the same size were detected by the sensory panel. These findings emphasize the importance of both particle size and surface morphology in causing gritty textures, offering insights for improving plant-based dairy alternatives.

1. Introduction

Plant protein concentrates and isolates are widely used in the development of dairy alternatives, but their low functionality, such as poor solubility, often poses a significant challenge (McClements & Grossmann, 2021; Sim et al., 2021). Undissolved particles can sediment or can be sensorially perceived as grainy, chalky, sandy, or gritty, resulting in texture defects in liquid or semi-solid dairy alternative formulations (Grossmann et al., 2021; McClements, 2020; McClements et al., 2019; Paul et al., 2020). While the precise meaning of each term may vary slightly depending on how it is defined in a given study, they essentially refer to the same phenomenon—the perception of particles in the liquid phase. However, for clarity and consistency throughout this paper, the term *gritty* is adopted as the standard descriptor. Beyond terminology, it is evident that texture remains a major challenge in the development of plant-based alternatives. According to the review by Paul et al. (2020) on plant-based milk analogs, improving texture (including stability) is a major focus area to enhance current plant-based milk alternatives. Studies show that consumers tend to prefer plant-based dairy alternatives that are smooth and creamy, while products perceived as gritty are generally liked less (Jaeger et al., 2024; Moss et al., 2022; Vaikma et al., 2025).

Plant protein powders produced on a small laboratory scale often exhibit good solubility, but their industrial-scale production employs harsher, cost-effective, and efficient processing conditions that can lead to protein denaturation and aggregation, resulting in decreased solubility (Burger et al., 2022a; Karaca et al., 2011; Moreno et al., 2020; Taherian et al., 2011). For example, various commercial products, such

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https://doi.org/10.1016/j.afres.2026.101699
Received 5 February 2025; Received in revised form 2 January 2026; Accepted 14 January 2026
Available online 15 January 2026
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as pea, wheat, rice, potato, sunflower, pumpkin, and canola, studied by Ebert et al. (2020), had protein solubility levels below 20 %. It is essential to differentiate between protein solubility and overall powder solubility. Protein concentrates, unlike isolates, can also contain substantial amounts of carbohydrates or fibers that may not dissolve (Jia et al., 2021). Consequently, any undissolved particles from the whole powder may cause an undesirable mouthfeel.

When assessing the solubility of ingredients in liquid end-applications, several critical factors must be considered. These include heat-treatment and homogenization processes that can alter the solubility properties of the material, and the pH level of the product. Commercial products typically undergo pasteurization to ensure microbiological stability, and heat treatment is often applied before fermentation in the production of plant-based yoghurt alternatives (Klost & Drusch, 2019). Fermented foods commonly exhibit an acidic pH of around 4–5 (Pua et al., 2022), a range at which the solubility of most plant proteins is at its lowest due to proximity to their isoelectric point. This increases the likelihood of larger aggregated particles forming, which can be perceived sensorially (Ma et al., 2022). For instance, pea samples analyzed by Burger et al. exhibited protein solubility of around 10 %, which increased to 20–55 % after homogenization. However, at an acidic pH of 4–5, protein solubility remained below 10 % even after homogenization (Burger et al., 2022a).

Research has demonstrated that sensory perception of particles depends on multiple properties, and not solely on particle size. Imai et al. (1995) studied microcrystalline cellulose particles in five dispersion matrices and found that grittiness was higher when the particles were bigger, their concentration was higher, and the viscosity of the matrix was lower. However, in other studies, rheological properties of the matrix had no influence on particle detectability and size perception (Engelen et al., 2005b; Petersson et al., 2013; Shewan et al., 2020). Tyle (1993) discovered that soft and round polyethylene particles up to 80 μm in a syrup were not detected as gritty, while hard and angular garnet particles were perceived as gritty at 11–22 μm. Engelen et al. (2005a) studied the effect of size, hardness, and shape of silica dioxide and polystyrene spheres ranging in average size from 2 μm to 230 μm in a vanilla custard dessert. The perception of roughness correlated strongly with particle size up to 80 μm, but at higher diameters roughness increased little, and hard particles with sharp edges also elevated the sensation. Appelqvist et al. (2015) conducted a study on carrot particles of varying sizes and morphologies. They showed that graininess—a sensation that can be compared to grittiness—was undetectable in samples with average sizes of 56 μm and 92 μm but clearly perceived at 225 μm. These examples indicate that although particle size and sensory perception are usually correlated, the perceivable size can vary. For example, Appelqvist et al. (2015) also mentioned the importance of particle hardness, concentration, and surface properties on sensory perception. In another investigation, Imai et al. (1999) prepared particles from various food items and analyzed them in the dry state; they demonstrated that properties like solubility and water absorption also influenced perception of grittiness by altering particle deformability and solidity. Likewise, plant protein powders absorb water, partially dissolve, or aggregate during food processing. Despite extensive research on particle properties, the sensory perception of plant protein particles in heat-treated and acidified water dispersions remains poorly understood, particularly in the context of dairy alternative production, thereby creating a knowledge gap.

Scanning electron microscopy (SEM) is commonly used to image the structure, shape, and surface morphology of powder particles (Kumar et al., 2022). In a study by Vogelsang-O'Dwyer et al. (2020), particles of a wet-fractionated fava bean protein isolate were found to be smooth, rounded, and shrunken, characteristics typical of spray-dried powders. Conversely, a dry-fractionated fava bean protein concentrate had more irregularly-shaped particles. He et al. (2020) also observed that oat flour particles displayed irregular morphology and were agglomerated, but after enzymatic treatment and spray drying, these particles became smaller and more spherical. Importantly, SEM imaging of protein powders in their native dry state cannot distinguish between particles that will dissolve and those that will remain undissolved, highlighting the limitations of dry-state measurements in understanding factors contributing to gritty mouthfeel in liquid end-products.

In our previous study, we investigated the powder solubility of several commercial plant protein powders using different methods: with and without heat treatment, at their native pH and pH 4.5 (Jakobson et al., 2023). The native pH of wet-fractionated plant protein powders is chosen by the manufacturer and is typically in the range of 6.0–7.7 (Jakobson et al., 2023). We also evaluated sensory texture attributes, including particle size and amount of particles, but only in the non-heat-treated samples at their native pH. Our findings confirmed the limited solubility of commercial protein powders and, more importantly, the presence of grittiness in most of these samples. However, correlations between solubility measured using different methods and sensory attributes were relatively weak, suggesting that other factors, such as particle size and morphology of undissolved particles, may contribute to the sensory perception.

In the present study, our objective was to investigate the effects of particle size and morphology on the sensory properties of plant protein powder dispersions. Diverging from previous research, we specifically examined the impact of undissolved particles in water-based dispersions subjected to heat treatment and acidification. We adopted this approach to simulate key steps relevant to the processing of dairy alternative end-products. Additionally, this study implemented a novel method by integrating sensory analysis with visual scoring of SEM images, allowing for a more detailed characterization of particle morphology while ensuring a straightforward and time-efficient process.

2. Materials and methods

2.1. Plant protein powders and preparation of liquid samples

Our primary objective was to characterize protein powder particles derived from various plant sources, with a preference for emerging crops, like legumes. To achieve this, we acquired 18 commercial plant protein powders, with most being isolates containing at least 80 % protein. However, commercial oat protein powders are currently available only as concentrates with a protein content of 56–59 %. Pea products dominated our selection due to their widespread commercial availability and use. The used plant protein powders and their protein content, as specified by the producers, are listed in Table 1. We characterized the nutritional composition and functional properties of the samples in more detail in our previous research (Jakobson et al., 2023).

Liquid samples of corresponding powders were prepared as 6 % dispersions in commercial filtered drinking water, then heat-treated at 85 °C for 15 min and cooled in an ice water bath. Then, the pH was either kept at its native level (without adjustment) or acidified to 4.5 using 10 % lactic acid. To summarize, we had three types of samples: dry powder, powder dispersion at native pH, and powder dispersion at pH 4.5, to which we refer, for example, as "Pea1", "Pea1 native", and "Pea1 pH 4.5", respectively. Native pH values ranged from 6.0 to 7.7. The samples were stored overnight at 4 °C and analyzed the following day. Two replicate dispersions were prepared from each powder for each

Table 1
Plant protein powder products and their declared protein content on the dry weight basis.

Sample	Protein, %	Sample	Protein, %	Sample	Protein, %
Chickpea1	89	Pea1	85	Pea7	80
Chickpea2	89	Pea2	85	Pea8	80
Fava bean	88	Pea3	84	Pea9	85
Mung bean	85	Pea4	80	Pea10	80
Oat1	59	Pea5	80	Soy	90
Oat2	56	Pea6	80	Wheat	82

condition. The same sample preparations were used for sensory, particle size, and SEM analyses. The 6 % dispersion of plant protein powders was selected to achieve protein concentrations comparable to those of conventional dairy products and in line with typical targets in the development of commercial plant-based dairy alternatives with good nutritional value (Craig & Brothers, 2021; Craig & Fresán, 2021). Commercial filtered water, thermal processing, and pH adjustment were used to simulate industrial conditions. Our previous research showed that results obtained after heating at native or acidified pH differed significantly from those without heating and acidification (Jakobson et al., 2023).

2.2. Particle size distribution

The particle size distribution was analyzed using two laser diffraction methods. Dry powders were measured using a PSA 1190 (Anton Paar, Graz, Austria) equipped with a dry jet dispersion unit, set to disperse the powder at an air pressure of 6 bar, vibrator duty cycle 75 % and frequency 78 %. The refractive index was set to 1.52. Liquid samples were measured at native pH and pH 4.5 using a Mastersizer 3000 (Malvern Instruments Ltd, Worcestershire, UK) equipped with a wet dispersion unit (Sun et al., 2019; Vogelsang-O'Dwyer et al., 2020; Yuan et al., 2021). Ultrapure water was used as the dispersant for native pH samples, while ultrapure water acidified to pH 4.5 with 80 % lactic acid was used for acidified protein powder dispersions. Refractive indexes of 1.33 and 1.45 were used for the dispersant and the protein, respectively, with the imaginary part set to 0.1. Samples were introduced into the dispersion unit using a disposable Pasteur pipette until the obscuration reached 5–10 %. The instrument was operated in slow stirrer mode at 500 rpm to avoid particle breakdown and ensure that measured particles were comparable to those evaluated by the sensory panel. Up to 10 measurements were recorded for each sample. The reconstruction mode used the Mie light scattering theory. The results are reported as volume-weighted D10, D50, and D90 diameters. These values indicate that particles smaller than the specified equivalent diameter represent 10 %, 50 %, and 90 % of the total volume of all particles, respectively.

2.3. Scanning electron microscopy

An environmental scanning electron microscope EVO LS15 (Zeiss, Oberkochen, Germany) was used to image the particles in selected samples. We investigated the morphology of the non-dissolved particles remaining after dispersing powders in water. The heat-treated dispersions at native and acidified pH were frozen in liquid nitrogen to preserve their structure and then lyophilized. The dried samples were evenly spread as a monolayer onto the SEM sample holder, which was pre-coated with double-sided adhesive carbon tape. To enhance conductivity, the samples were sputter-coated with a 1.5 nm layer of gold/palladium (80:20) and then imaged at 15 kV, with magnifications of × 200 and × 500, using the extended pressure secondary electron detector at a pressure of 70 Pa.

2.4. Sensory assessments

Sensory analysis serves as an objective method in which a trained panel acts as a calibrated instrument, using human perception as an instrumental metric to quantify the sensory characteristics of a product (Sipos et al., 2021). According to ISO 8586:2023 (ISO, 2023), an expert sensory assessor is defined as a person "with a demonstrated sensory sensitivity and with considerable training and experience in sensory testing, who is able to make consistent and repeatable sensory assessments of various products". In this study, a previously trained panel was employed to ensure the repeatability and reliability of sensory tests (Djekic et al., 2021; Sipos et al., 2021). All assessors were recruited from AS TFTAK in accordance with ISO 8586:2023 standard (ISO, 2023).

Two different sensory tests were conducted: a texture (particle size) evaluation of powder dispersions in water by mouthfeel, involving nine assessors, and a SEM image evaluation by appearance (particle morphology), involving ten assessors. The panels comprised of different assessors, some of whom were trained in texture analysis and some in appearance. This aligns with the standard practice in sensory research, which typically involves a trained panel of 8 to 12 members (Djekic et al., 2021). Prior to the evaluation sessions, each panel underwent additional trainings using specific examples from the study to familiarize themselves with the samples and clarify terminology where necessary. All participants from the sensory panel gave consent to take part in the experiment and were informed in advance of the procedures of the study. Participation was voluntary, and assessors could withdraw from the test at any time.

The sensory tests were conducted in accordance with ISO 6658:2017 (ISO, 2017), incorporating standard practices such as blinding samples with random three-digit codes, using structured scales to assess differences, and conducting evaluations in a specialized sensory room. This room was designed in compliance with ISO 8589:2007 (ISO, 2007) to eliminate any external factors that might influence the results. Samples were presented to assessors in a sequential monadic pattern in randomized order following the Williams Latin Square design to avoid the effect of presentation (Macfie et al., 1989). All sample preparations were evaluated in two replicate sessions to enhance discrimination accuracy (Peltier et al., 2018). Each session lasted up to 30 min each, which aligns with the common approach of keeping sensory sessions under two hours to reduce sensory fatigue (Djekic et al., 2021). Assessors were asked to evaluate the samples on a 10-point scale (0–9), which aligns with common practices in sensory studies (Djekic et al., 2021). Scale points 0, 1, 5, and 9 had descriptors specific to the attribute being evaluated (Table 2). Data collection was performed using RedJade sensory software (RedJade Sensory Solutions LLC, Martinez CA, USA).

2.4.1. Particle size evaluation

For particle size evaluations, 25 mL of each sample dispersion was dispensed into a 40 mL transparent plastic cup. All samples were served and evaluated at room temperature. The water dispersions of the samples were mixed vigorously before pouring to avoid stratification. Between samples, palate cleansing was performed using potable water (Saku Läte OÜ, Estonia) and unsalted crackers (Pladis LTD, London, UK). The specific definition of the assessed particle size attribute is shown in Table 2.

2.4.2. Visual assessment of SEM images

Studies evaluating particle morphology using SEM often assign qualitative shape descriptors, such as "smooth" or "irregular", but do not quantify these characteristics. Uniquely, we employed a sensory panel to visually assess SEM images and quantify particle morphology. Our decision against relying on software-based image analysis was based on its limitations: simple automated image analysis measures only two-dimensional contours of particles, while advanced techniques are labor intensive and their results depend heavily on other factors like image quality and analysis algorithms (Nayak et al., 2019). Whereas a human eye can infer three-dimensional shapes and describe surface features more precisely from a single image. Human assessors can also integrate macrostructural features, such as edges and protrusions, with microstructural features, such as fine texture and irregularities, providing a holistic interpretation of particle morphology. Thus, this less time-consuming approach enables a more nuanced evaluation of morphological characteristics, specifically angularity, roughness, and heterogeneity. Further, the approach used in this study makes a significant contribution to the limited literature on the sensory evaluation of images. Some publications on image assessment by sensory panels have focused on evaluations of broccoli preservation (Garitta et al., 2013), the complexity of fruit and vegetable mixes (Mielby et al., 2012), consumer acceptance of raw breast fillets (Kuttappan et al., 2012), and facial skin roughness (Donofrio et al., 2016), all of which were considered during

Table 2
Sensory attributes, definitions, and scale descriptions.

Attribute	Definition	Scale descriptions			
		0	1	5	9
Size	Describes the perceived size of particles. A protein solution was included as a reference. Evaluated based on mouthfeel.	Particles missing	Very small	Moderate	Very large
Roughness	Describes the unevenness, coarseness of the particle surface. Photos included as references. Evaluated based on SEM images.	Completely smooth	Slightly rough	Half-smooth/ rough	Completely rough
Angularity	Describes the irregularity, angularity of particle shape. Photos included as references. Evaluated based on SEM images.	Completely circular	Slightly angular	Half-circular/ angular	Completely angular
Heterogeneity	Describes how dissimilar are shapes and sizes of all particles. Photos included as references. Evaluated based on SEM images.	Completely homogenous	Slightly heterogenous	Half-homogenous/ heterogenous	Completely heterogenous

the development of our methodology.

SEM images were shown on a tablet screen, each photo sized 82 mm x 122 mm. For each sample, the perceived roughness of the particle surfaces, as well as the angularity and heterogeneity of the particle shapes, were assessed (Table 2). Two images from different areas were provided to assess each attribute, as previous training sessions indicated that the result may depend on the image, particularly for heterogeneous samples. Higher magnification images (× 500), as shown in Figs. 1A and B, were used to assess roughness and angularity, allowing for a more precise impression of the morphology, while lower magnification images (× 200) from the same positions, as shown in Figs. 1C and D, were used to assess heterogeneity, covering a larger area. The panel was asked to assess each attribute based on the overall impression of all particles visible in the images, excluding fiber-like fragments. The assessment was performed in two replicate sessions.

2.5. Statistical analysis

Data analysis and visualization were performed in R version 4.3.0 (The R Foundation for Statistical Computing, Vienna, Austria). The data are reported as means with standard deviations. Locally estimated scatterplot smoothing (LOESS) regression, with a span of 1.1, was applied to model D90 as a function of sensory size, while the R package "segmented" version 2.0-1 was used to verify the presence and location of a breakpoint in this model. Multiple linear regression was applied to identify a relationship between the sensory perception of size, particle morphology, and particle size distribution. The quality of these models was evaluated using the R package "performance" version 0.10.8. Correlations were calculated as Spearman ρ using the R package "correlation" version 0.8.4.

3. Results and discussion

3.1. Particle size distribution

Particle size distribution is a property that is related to powder solubility and influences the mouthfeel and stability of a liquid end-product that contains the powder (Barbosa-Canovas et al., 2005). Given that plant protein powders absorb water and partially dissolve, we measured them in their dry state and in a liquid environment. Furthermore, protein solubility is highly dependent on pH level. Fermented dairy alternatives, such as plant-based drinks or yogurt-style products, typically have an acidic pH of around 4.5. The production process commonly includes pasteurization to ensure microbiological safety and extend shelf-life. Consequently, we incorporated these processing conditions, including acidification and pasteurization, into our liquid sample preparation protocol (Jakobson et al., 2023).

A strong correlation was observed between the D50 and D90 values

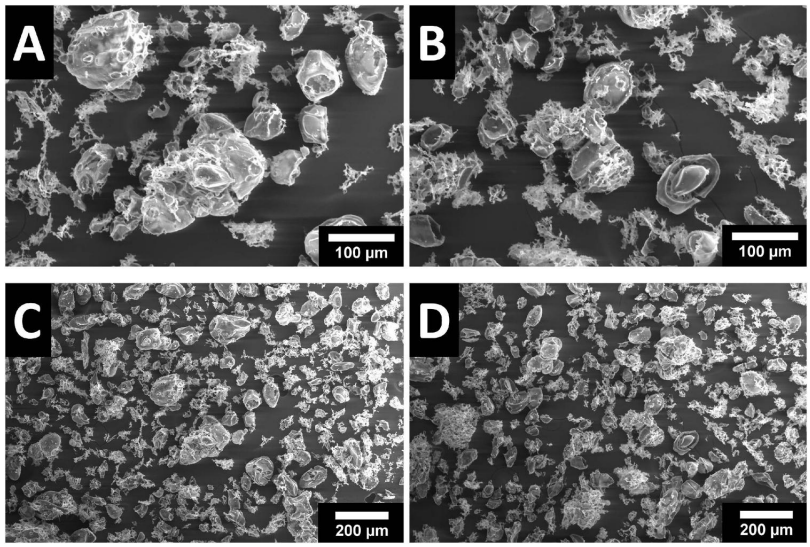

Fig. 1. An example of presentation of scanning electron microscopy images (Pea4) for visual scoring by the assessors. Images A and B were made at higher magnification, while C and D at lower magnification. Images A and C were made from one position, while B and D were from another. The powder was dispersed in water at a 6 % concentration, heat-treated at 85 °C for 15 min, acidified to pH 4.5, then lyophilized.

(ρ = 0.92), and the same conclusions presented in this paper could be drawn using either D50 or D90 values. Fidaleo et al. (2017) showed a strong correlation between D90 and the sensory perception of particles. Therefore, we decided to focus on the D90 values, which are summarized as a ladder plot in Fig. 2, with additional data available in Appendix Tables A1–A2.

The D90 values of all studied samples measured in the dry state were 114 μm on average, with a range spanning from 56 μm to 235 μm. These extrema were observed in the pea samples, but as shown in Fig. 2, large differences were also found among the oat samples (109–226 μm). Spray drying is a typical method employed in commercial production of wet-fractionated plant protein powders, with the particle size distribution of the resulting powders influenced by both the drying equipment and processing conditions (Burger et al., 2022b). However, samples Pea2 and Pea6 were additionally ground after drying and, thus, had substantially smaller particles compared to the other pea samples.

In contrast to dry-state measurements, the particle size distribution of powders dispersed in water and heat-treated changed drastically. The D90 values of most samples increased, as evident from the slopes of the lines in Fig. 2, except for Pea1, Pea3, and Pea9, which showed a decrease. Excluding soy and wheat, the particle sizes at native pH ranged from 75 μm to 375 μm. At pH 4.5, this range widened to 73–493 μm, although acidification had little influence on the size in half of the samples, as indicated by the horizontal lines in Fig. 2. For comparison, Osen et al. (2013) reported D90 values of 109–344 μm for three commercial pea protein isolates dispersed in 1-butanol, and Zhang et al. (2024) reported D90 values of 120–470 μm for fourteen commercial soy protein isolates dispersed in water. Surprisingly, our soy sample, which was highly soluble at native pH and insoluble at acidic pH (Jakobson et al., 2023), formed large visible lumps of 1 mm regardless of pH. The wheat sample formed aggregates of 1 mm at native pH, increasing to 2 mm under acidic conditions, despite its higher solubility at pH 4.5 (Jakobson et al., 2023).

The discrepancies in particle size distributions observed in protein powders between the dry and liquid states at two different pH levels can be attributed to varying physico-chemical properties. Plant protein powders absorb water and swell, they can partially dissolve, aggregate, and form gels due to heat-treatment or pH change (Mession et al., 2013). The chosen pH of 4.5 is a typical isoelectric point of plant globulins (Tang et al., 2023), at which they carry no net electrical charge and electrostatic repulsion between molecules is at its weakest, allowing formation of large protein aggregates due to hydrophobic interactions (Yang et al., 2024). We have previously reported that water holding capacity of commercial protein powders was in the range of 0.9–6.3 g water g^{-1} powder and solubility at native pH was 5–94 %, while at pH 4.5 it decreased to 0–39 % (Jakobson et al., 2023). The variability of these processes was reflected in much higher standard deviations recorded in liquid samples, particularly for soy and wheat, while replicates of the dry measurements were almost identical. Notably, the results from measurements conducted under different conditions were not strongly correlated, with a Spearman ρ of 0.69 between native and acidic pH levels, but only 0.18 between the dry state and the liquid state at both pH values. Our findings differ somewhat from those reported in the literature. Typically, other studies use methods that minimize dissolution, aggregation, or other particle changes, such as dry dispersion or in organic solvents, or if dispersed in water, then at native pH without further treatment. However, the lack of correlation between our results suggests that even if a plant protein powder exhibits small particles in water at native pH or in the dry state, its suitability for acidic dairy alternatives may not be guaranteed. Lowering the pH may cause particles to aggregate, leading to sensory defects like grittiness. This reiterates the importance of using sample preparation conditions that accurately reflect the end application when assessing the suitability of protein ingredients for product development.

3.2. Sensory perception of particle size

To differentiate between the instrumentally measured particle diameter D90 and the particle size perceived by the assessors, we refer to the latter as sensory size. Sensory size scores at native and acidified pH are summarized in Fig. 3. Acidification generally amplified particle perception across the samples, including previously undetectable ones, likely due to structural changes or aggregation. At native pH, the perceived sensory particle size ranged from no particles detected (0.0 ± 0.0; number of assessors $n = 9$) to moderate-sized particles (6.1 ± 0.8; $n = 9$), whereas for acidified samples, the range extended from very small particles (1.1 ± 0.2; $n = 9$) to very large particles (9.0 ± 0.0; $n = 9$). For pea samples, sensory sizes at native pH ranged from 0.0 ± 0.0 to 2.3 ± 0.6 ($n = 9$), whereas acidification expanded the range from 1.1 ± 0.2 to 5.8 ± 0.5 ($n = 9$). Pea samples showed diverse responses to acidification, with some exhibiting dramatic increases (e.g., Pea9, from 0.0 ± 0.0 to 5.8 ± 0.5; $n = 9$) while others remained relatively consistent (e.g., Pea1, from 1.3 ± 0.6 to 1.1 ± 0.2; $n = 9$). The soy sample showed the largest increase, from 0.0 ± 0.0 at native pH to 8.4 ± 0.5 ($n = 9$) under acidic

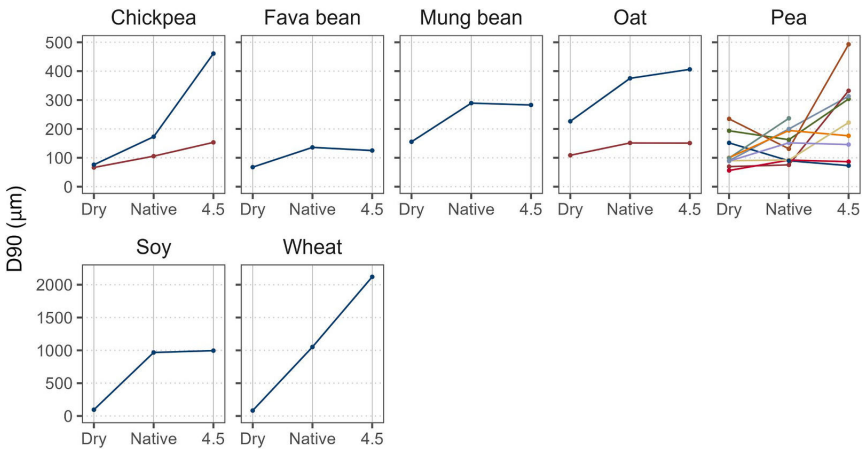

Fig. 2. Ladder plot of average volume-weighted particle diameters (D90) of plant protein powders measured instrumentally in the dry state and as 6 % water dispersions after heat-treatment (85 °C for 15 min), at native pH and pH 4.5. Colored lines are used to guide the eye and represent different products from the same crop. Note the different y-axis scale for soy and wheat.

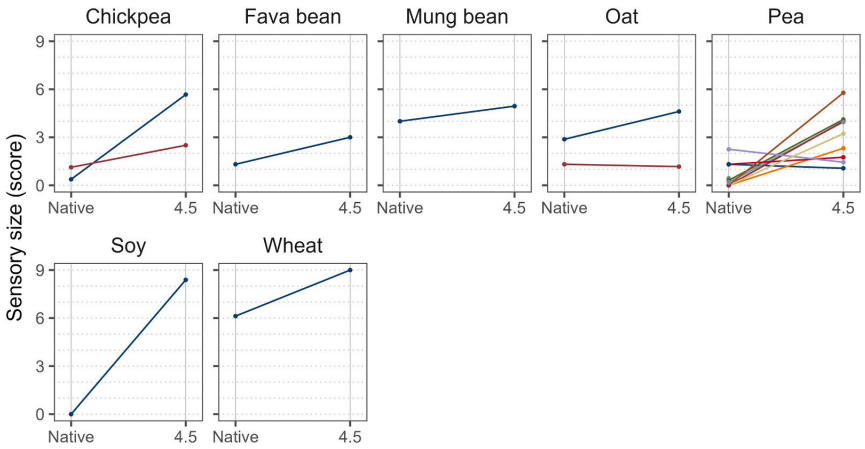

Fig. 3. Ladder plot of average particle size of plant protein powders measured sensorially as 6 % water dispersions after heat-treatment (85 °C for 15 min), at native pH and pH 4.5. Colored lines are used to guide the eye and represent different products from the same crop.

conditions. Interestingly, soy at native pH had a large D90 of 968 μm, but the lack of sensory perception may be related to the highly hygroscopic nature of this specific powder as it formed soft cloudy clumps. These particles may have been softer than human mouth mycosa and therefore the panelists were not able to detect them in the mouth (Appelqvist et al., 2015; Shewan et al., 2020). Overall, these data highlight that pH and heat treatment are critical factors that impact the sensory perception of particle size in the case of protein powders.

Previous research has shown that sensory perception of particles is influenced by multiple factors, including their physical size, shape, material properties, interactions with the surrounding matrix, as well as environmental conditions. A study using microcrystalline cellulose (D50 6–79 μm) found that particle concentration was the primary factor contributing to grittiness, followed by dispersion medium viscosity and particle size. In that study, assessors detected grittiness in a 0.4 % aqueous suspension at particle sizes as small as 10 μm, but in a 0.2 % suspension, only larger particles of 25–80 μm size caused grittiness. In hard gels, detection thresholds increased to 3 % particle concentration (Imai et al., 1995). Similarly, hard and irregular rye bran particles of diameters in the range of 20–180 μm were perceived as gritty in starch gels at concentrations as low as 0.1–0.3 % (Petersson et al., 2013). The texture defect of sandiness in dairy ice cream is caused by hard and sharp lactose crystals that grow larger than 15–30 μm in diameter, depending on their concentration (Livney et al., 1995); this sensation can also be compared to grittiness. The results of a study in an acidic environment of yoghurt followed these trends, specifically highlighting the masking effect of viscosity and the amplifying effects of hardness and particle concentration (Mantilla et al., 2020). In our study, we selected particle concentration and environmental conditions to reflect those found in industrial liquid dairy alternatives. Given the typically low solubility of plant protein powders (Jakobson et al., 2023), the concentration of particles in our dispersions (6 %) was well above the reported detection thresholds, in addition, the low viscosity of our dispersions offered little masking effect. As we previously reported, the median viscosity of these powders dispersed at an even higher concentration of 9 % was only 30 mPa s (Jakobson et al., 2023). For comparison, the masking effect of viscosity in the study by Appelqvist et al. (2015) was observed at values above 100 mPa s. Instrumentally measured particle sizes in our samples (D90 73–493) were in the range or larger than reported in the literature, so the particles should have been easily perceptible. Yet, at native pH, half of the samples had sensory size scores below 0.5, necessitating further investigation to understand this discrepancy.

3.3. Correlation between instrumentally measured and sensorially perceived particle size

Our findings revealed no correlation between sensory size, evaluated as powder dispersions in water, and D90 measured in the dry state, with ρ values of −0.04 at native pH and 0.2 at pH 4.5, supporting our hypothesis that particle size must be measured under conditions relevant to the intended food application. Plotting sensory size against D90 measured in the liquid state revealed a clear trend, but surprisingly nonlinear (Fig. 4). To emphasize the change in the trend, we added a LOESS regression line to the plot. A linear relationship was found between instrumental particle size and sensory size when D90 was roughly above

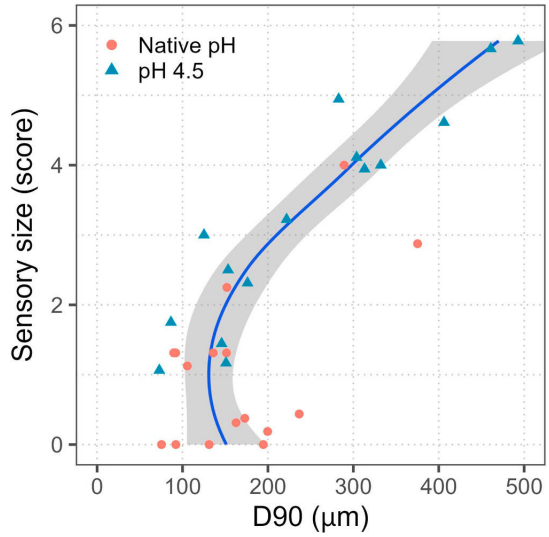

Fig. 4. Sensory perception of particle size as a function of instrumentally measured volume-weighted particle size distribution (D90) in 6 % water dispersions of plant protein powders after heat treatment (85 °C for 15 min), at native pH and pH 4.5. The blue line shows LOESS regression, and the gray band shows its 95 % confidence interval. The extreme values for soy and wheat were excluded.

200 μm and the sensory score was above 2, regardless of pH. However, this trend was absent for smaller particles. Moreover, some samples with similar D90 values showed considerably different sensory sizes. For instance, fava bean at pH 4.5 and Pea9 at native pH both had D90 values around 130 μm, yet the sensory size of the former was rated as 3, while the latter was not perceived by the sensory panel at all. Likewise, Pea2 and Pea4 at native pH had sensory sizes of 0.0 ± 0.0 (number of assessors $n = 9$) despite their D90 values being 75 μm and 195 μm, respectively. A possible explanation for this leveling-off trend is that the particle sizes approached the threshold of sensorial detectability, which in our case was around 73 μm, the lowest measured D90 value. Since the variance of sensory scores did not increase with smaller particle sizes, this means that the sensory panel was able to score the samples with confidence regardless of particle size, rendering this explanation insufficient to describe the trend. Given the evidence in the literature that particle physical properties influence their perception, the trend we observed may be rather related to factors like hardness, water absorption, solubility, and surface morphology. For instance, Appelqvist et al. (2015) found that soft and deformable carrot cell wall particles were not detectable in custard matrices when particle sizes were in the range of 30–400 μm. Another study in custards showed that round and soft polystyrene particles were less detectable than sharp and rigid silica dioxide particles of similar size. Furthermore, silica dioxide particles of size 80 μm (D50) were perceived as larger than 230 μm polystyrene particles (Engelen et al., 2005b). We can reasonably assume that our plant-protein powder particles, after sample preparation under conditions close to those used in industrial end applications, were relatively soft. We also found no association between powder solubility and D90, which prompted us to investigate the morphological properties of the particles using SEM.

3.4. Visual assessment of SEM images

In Fig. 5, we present a selection of SEM images of particles from diverse samples with varying D90 and sensory sizes, while the quantitative results are shown in Table 3, along with corresponding D90 and sensory sizes for easier comparison. The angularity attribute describes larger particle features, such as edges and protrusions, whereas roughness reflects finer, microstructural details on the particle surface. Heterogeneity refers to the variation in shapes of all visible particles. Note that because the whole sample dispersions were lyophilized, SEM images also contained a relatively small amount of dissolved dry matter, which can be differentiated from the undissolved particles as having a fluffy or fiber-like appearance, typically seen in lyophilized proteins (Beech et al., 2015). The presence of dissolved dry matter had some influence on the results, but to minimize it, the panelists were instructed to ignore such fragments during the assessment.

As seen in the SEM image (Fig. 5), Pea4, a sample with a relatively large D90 and 0.0 ± 0.0 (number of assessors $n = 9$) sensory size at native pH, had uniformly round and smooth particles with minimal

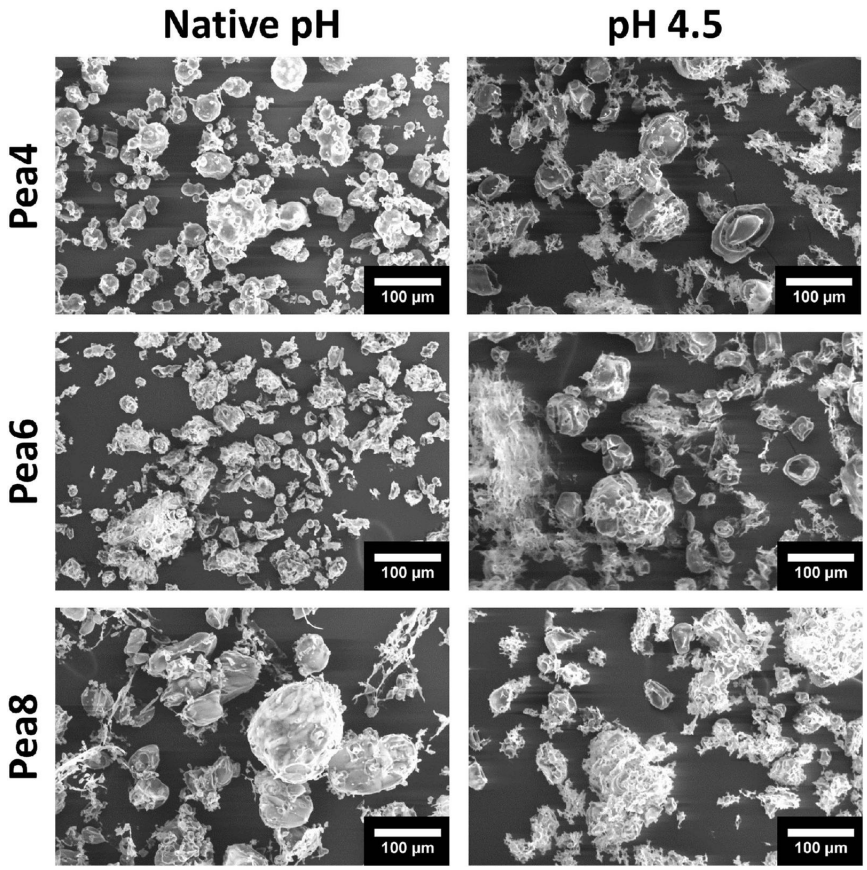

Fig. 5. Examples of scanning electron microscopy images of protein powders. The sensory scores assigned to these images are shown in Table 3. The powders were dispersed in water at a 6 % concentration, heat-treated at 85 °C for 15 min, then either kept at native pH or acidified to pH 4.5, and then lyophilized.

Table 3
Average sensory scores with standard deviations for particle morphology attributes (number of assessors = 10, replicated twice) evaluated based on scanning electron microscopy images. Corresponding sensory (number of assessors = 9, replicated twice) and instrumentally measured sizes (number of replicates = 10) are also shown here for comparison.

Sample	pH	Roughness	Angularity	Heterogeneity	Sensory size	Instrumental size D90, μm
Pea4	Native	2.9 ± 0.9	2.3 ± 1.0	2.8 ± 1.0	0.0 ± 0.0	195 ± 8
Pea8	Native	3.0 ± 0.7	2.8 ± 0.8	4.3 ± 0.9	0.0 ± 0.0	92 ± 2
Pea5	Native	4.3 ± 0.7	5.0 ± 0.9	4.8 ± 0.9	0.4 ± 0.4	237 ± 4
Pea1	4.5	6.5 ± 0.7	6.4 ± 0.8	4.0 ± 0.9	1.1 ± 0.2	73 ± 3
Oat2	4.5	7.7 ± 0.9	6.4 ± 1.0	7.9 ± 0.9	1.2 ± 0.3	151 ± 6
Pea6	Native	6.1 ± 0.7	6.3 ± 0.7	4.2 ± 0.9	1.3 ± 0.5	92 ± 1
Pea1	Native	8.0 ± 1.0	7.0 ± 0.8	7.3 ± 1.0	1.3 ± 0.6	90 ± 2
Oat2	Native	8.5 ± 0.7	8.2 ± 0.8	8.3 ± 0.7	1.3 ± 0.5	151 ± 10
Pea6	4.5	3.7 ± 0.6	4.5 ± 1.0	5.2 ± 0.9	1.8 ± 0.4	86 ± 2
Pea4	4.5	5.1 ± 0.9	4.5 ± 1.0	5.9 ± 1.0	2.3 ± 0.5	176 ± 18
Pea8	4.5	7.7 ± 0.9	6.6 ± 0.9	6.6 ± 0.5	3.2 ± 0.8	222 ± 33
Pea9	4.5	7.0 ± 0.8	6.1 ± 0.8	6.8 ± 1.0	5.8 ± 0.5	493 ± 54
Soy	4.5	8.2 ± 0.8	7.3 ± 0.7	6.9 ± 1.0	8.4 ± 0.5	996 ± 359
Wheat	4.5	8.8 ± 0.4	8.5 ± 0.7	8.2 ± 0.8	9.0 ± 0.0	2120 ± 618

aggregation, reflected in low visual scores for roughness (2.9 ± 0.9; n = 10), angularity (2.3 ± 1.0; n = 10), and heterogeneity (2.8 ± 1.0; n = 10) (as shown in Table 3). After acidification to pH 4.5, despite a slight decrease in D90, the sensory size increased from 0.0 ± 0.0 to 2.3 ± 0.5 (n = 9), and the particles appeared more rough, angular, and inhomogeneous in the SEM images, with respective scores of 5.1 ± 0.9, 4.5 ± 1.0, and 5.9 ± 1.0 (n = 10). In contrast to Pea4, Pea6 at native pH had a twice smaller D90, but the particles were detected sensorially. In the SEM images, these particles looked quite rough (6.1 ± 0.7; n = 10) and angular (6.3 ± 0.7; n = 10). Pea8 at native pH exhibited a similarly small D90 as Pea6, but its particles were not perceived sensorially. This corresponded with SEM images showing particles that were smooth and round, with roughness (3.0 ± 0.7; n = 10) and angularity (2.8 ± 0.8; n = 10) similar to Pea4. After acidification to pH 4.5, Pea8 particles showed a notable increase in both instrumental and sensory size and appeared very rough and angular, with scores of 7.7 ± 0.9 and 6.6 ± 0.9 (n = 10), respectively. These findings clearly indicate that angular, rough, and heterogeneous undissolved plant protein powder particles are more likely to be detected sensorially, whereas smooth and round ones are less perceptible.

Overall, all visually assessed samples were highly distinguishable, with scores spanning a wide range. However, the three assessed attributes—angularity, roughness, and heterogeneity—were highly intercorrelated (ρ = 0.75–0.97), indicating that more angular particles also tended to have rougher surfaces on a smaller scale and more varied shapes. This correlation may also suggest that assessors had difficulty distinguishing between what feature on the particle surface should be considered as large-scale (angularity) or small-scale (roughness), particularly given the significant variation in particle sizes in the images. Yet, no correlation was observed between D90 and the three attributes (ρ = 0.25–0.35), indicating that smaller particles were not necessarily less angular, rough, or heterogeneous, thereby refuting this hypothesis.

3.5. Relationship between the particle morphology, size distribution, and sensory size perception

We applied multiple linear regression to investigate how both particle size distribution and surface morphology influence sensory perception of particle size. The trend in Fig. 4 suggests that the data has two different regions, so we confirmed the existence of a breakpoint using a Score test ($p < 0.05$). A segmented linear model was then fitted, and the location of the breakpoint was estimated to be at sensory size of 1.75 (95 % confidence interval 0.98–2.52). After this, as shown in Table 4, we fitted separate models for particles with sizes above or below the breakpoint to predict sensory size from D90 alone or in combination with angularity or roughness. The reciprocal of heterogeneity served as a weight in regression, reducing the influence of more heterogeneous

Table 4
Models fitted to the sensory and instrumental data and their corresponding performance. Here, β_{0-2} are the regression coefficients, AICc—corrected Akaike Information Criterion.

Model	p-value	AICc	Adjusted R^2
Larger particles with sensory size ≥ 1.75			
$Sensory\ size = \beta_0 + \beta_1 \log(D90)$	0.001	28.6	0.95
$Sensory\ size = \beta_0 + \beta_1 \log(D90) + \beta_2(Roughness)$	0.008	58.2	0.93
$Sensory\ size = \beta_0 + \beta_1 \log(D90) + \beta_2(Angularity)$	0.009	58.5	0.93
Smaller particles with sensory size < 1.75			
$Sensory\ size = \beta_0 + \beta_1 \log(D90)$	0.232	24.3	0.10
$Sensory\ size = \beta_0 + \beta_1 \log(D90) + \beta_2(Roughness)$	0.002	15.6	0.89
$Sensory\ size = \beta_0 + \beta_1 \log(D90) + \beta_2(Angularity)$	0.001	13.3	0.92

samples on model fitting. To determine the best model, we used p-values, the Akaike Information Criterion corrected for small sample sizes (AICc), and adjusted R^2. In the case of R^2, a higher value means that the model explains more of the variance in the data, but for AICc, a lower value shows that it explains the data with fewer parameters.

For larger particles, the adjusted R^2 values were nearly equal across the three models (Table 4). However, models incorporating angularity or roughness had much higher AICc values, indicating that these additional variables did not improve the base model, which included only D90. The p-values for angularity and roughness coefficients in these models were also not statistically significant ($p > 0.05$). Therefore, for larger particles, their size distribution is the primary factor influencing sensory perception of their size, whereas particle surface morphology is not important. For smaller particles, the model with only D90 was not statistically significant and exhibited a very low R^2 and high AICc, suggesting that it failed to explain the data. In contrast, the addition of either roughness or angularity greatly improved the model. Both of these models were statistically significant, including the roughness and angularity coefficients ($p < 0.05$), and performed comparably due to the high correlation between angularity and roughness (ρ = 0.98). Nevertheless, the model incorporating angularity had slightly lower AICc, making it the best of the three. This confirms that for small plant protein particles—specifically those with a sensory size below 1.75 and D90 below 200 μm—their sensory perception is more dependent on their surface morphology than size distribution.

Our results align with the findings of Tyle (1993), who reported that the threshold for sensory detection of angular and hard particles was significantly lower compared to round and soft ones. On the other hand, the inability of the D90-only model to explain the data for our smaller particles (< 200 μm) contradicts the research by Engelen et al. (2005a), which showed a strong and almost linear relationship between particles in the range of 4–213 μm (D90) and their sensory size perception. This discrepancy may be related to the hardness of the particles, as that study

utilized hard and highly angular silicon dioxide particles, but plant protein powders used in our study are assumed to be soft and partially dissolved or aggregated. Several studies have reported the influence of particle hardness on sensory perception. In a study by Shewan et al. (2020), agar microgel particles in water suspensions became more sensorially detectable and their size felt bigger as the particle storage modulus increased. Appelqvist et al. (2015) observed that carrot cell wall particles with a D90 of 190 μm were not perceived as grainy, whereas carrot cell clusters with a D90 of 440 μm were clearly grainy—a sensation that can also be compared to grittiness. They attributed this difference in perception to the hardness of the particles rather than their size, although they did not quantify the hardness properties. However, the most notable study was conducted by Imai et al. (1999), which similarly to us, demonstrated that sensorial recognition of individual particles of various foods cannot be fully expressed by their physical properties individually, but rather in combination. Their multiple linear regression model, which included particle density, solubility, water absorption rate, deformation resistance, and fluctuation in friction coefficients, had a multiple correlation coefficient of 0.88—much higher than the correlation coefficients of models with single properties. We can hypothesize that particle surface morphology, which we assessed as roughness and angularity, can be related to particle friction properties, and thus our results support their conclusion. Nevertheless, we add to that knowledge that the abovementioned physical properties of particles may be important only near the threshold of their sensory detection, but for larger particles, their physical size may be the predominant attribute.

4. Conclusions

We investigated the factors influencing the sensory perception of grittiness caused by plant protein powders, a common issue in liquid dairy alternatives. We found that sample preparation must follow typical production steps of the target end-product to generate any relevant data and that powder particle size measurement in the dry state does not predict the sensorial size perception. We identified a relationship between the sensorially perceived particle size and instrumentally measured particle size in plant protein powders dispersed in water, but it is non-linear, and for particles smaller than approximately 200 μm (D90), their surface morphology becomes more important than just physical size. Particles with rough surfaces and angular shapes elicit a stronger size perception in the mouth, whereas smooth and round particles of the same size may not be perceived at all. We developed a unique method for assessing surface properties of particles, combining SEM imaging with visual grading by a sensory panel. This method not only provided numerical results describing particle morphology but also advanced the methodological framework for sensory assessments of imagery in general. All in all, the surface morphology of plant protein particles is crucial in determining the texture of the final product, and understanding and controlling this morphology is essential for tailoring the textural characteristics of liquid dairy alternative products.

Ethical statement

This study was conducted in accordance with the ethical principles outlined in The Code of Ethics of the World Medical Association (Declaration of Helsinki) and the Principles of Academic Ethics by Tallinn University of Technology (Estonia). Since sensory research of food in Estonia does not require formal ethical approval, an official approval number is not available. This study did not involve collecting sensitive personal data. Nevertheless, responsible data management was implemented to prevent participant identification and ensure confidentiality, as per the General Data Protection Regulation (GDPR). Participants for the sensory tests were selected from an internal pool of highly trained evaluators who volunteered for sensory assessments. Before the study began, participants were informed of its purpose and procedures and provided written consent. Participation was voluntary, and panelists were free to withdraw from the tests at any time. All panelists were in good health and had no known allergies to the studied materials. Participants were assured of the confidentiality of their data. The informed consent form, digitally signed by all participants, is available in the supplementary file.

Funding details

Financial support for this research was provided by the European Regional Development Fund (ERDF) and the Estonian Research Council via project RESTA16.

CRediT authorship contribution statement

Kadi Jakobson: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Aleksei Kaleda:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Helen Vaikma:** Writing – original draft, Methodology, Investigation, Formal analysis. **Andres Sats:** Writing – review & editing, Resources, Investigation. **Sirli Rosenvald:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Tiina Kriščiunaite:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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.

Acknowledgments

The authors are grateful to Grete Metsoja for assistance in organizing the sensory assessments, to Valdek Mikli for assistance with SEM imaging, to Karel Talvistu for measurement of particle size distributions in the dry state, and to Marianna Surženko for designing the graphical abstract. We thank three anonymous reviewers for critically evaluating the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2026.101699.

Appendix

Appendix Table A1
Particle size distributions of plant-protein powders measured in the dry state shown as a mean volume-weighted diameter with standard deviation (number of replicates = 2).

Sample	D10, μm	D50, μm	D90, μm
Chickpea1	18 ± 1	43 ± 0	76 ± 0
Chickpea2	15 ± 0	38 ± 0	66 ± 0
Fava bean	12 ± 0	35 ± 0	68 ± 1
Mung bean	19 ± 0	64 ± 0	155 ± 0
Oat1	9 ± 1	56 ± 1	226 ± 18
Oat2	9 ± 0	44 ± 0	109 ± 0
Pea1	15 ± 3	74 ± 1	152 ± 0
Pea2	9 ± 0	38 ± 0	70 ± 0
Pea3	20 ± 1	74 ± 0	194 ± 0
Pea4	21 ± 0	50 ± 0	100 ± 0
Pea5	12 ± 0	48 ± 0	99 ± 0
Pea6	8 ± 0	31 ± 0	56 ± 0
Pea7	17 ± 1	44 ± 0	88 ± 0
Pea8	21 ± 0	47 ± 0	90 ± 0
Pea9	28 ± 0	106 ± 0	235 ± 0
Pea10	17 ± 0	46 ± 0	90 ± 0
Soy	18 ± 1	50 ± 0	96 ± 0
Wheat	7 ± 0	36 ± 0	83 ± 0

Appendix Table A2
Particle size distributions of plant protein powders measured in 6 % water dispersions after heat-treatment (85 °C for 15 min), at native pH and pH 4.5 shown as a mean volume-weighted diameter with standard deviation (number of replicates = 10).

pH	Native			4.5		
Sample	D10, μm	D50, μm	D90, μm	D10, μm	D50, μm	D90, μm
Chickpea1	24 ± 2	83 ± 4	173 ± 5	37 ± 4	129 ± 19	461 ± 71
Chickpea2	13 ± 1	51 ± 2	106 ± 3	16 ± 1	60 ± 27	153 ± 122
Fava bean	16 ± 0	62 ± 1	136 ± 2	27 ± 2	62 ± 3	125 ± 6
Mung bean	32 ± 0	133 ± 3	289 ± 6	27 ± 1	121 ± 2	283 ± 9
Oat1	14 ± 1	102 ± 8	375 ± 58	17 ± 2	112 ± 12	406 ± 51
Oat2	17 ± 1	62 ± 2	151 ± 10	16 ± 1	61 ± 1	151 ± 6
Pea1	9 ± 1	42 ± 1	90 ± 2	10 ± 0	31 ± 2	73 ± 3
Pea2	14 ± 0	37 ± 0	75 ± 1	19 ± 4	93 ± 20	332 ± 64
Pea3	25 ± 2	73 ± 5	163 ± 10	16 ± 1	87 ± 6	304 ± 60
Pea4	25 ± 4	92 ± 5	195 ± 8	15 ± 3	75 ± 6	176 ± 18
Pea5	22 ± 1	115 ± 2	237 ± 4	-	-	-
Pea6	9 ± 0	45 ± 0	92 ± 1	9 ± 1	41 ± 2	86 ± 2
Pea7	24 ± 1	70 ± 2	152 ± 8	21 ± 1	66 ± 1	146 ± 3
Pea8	11 ± 0	36 ± 2	92 ± 2	13 ± 0	63 ± 4	222 ± 33
Pea9	18 ± 1	60 ± 4	131 ± 8	29 ± 6	122 ± 11	493 ± 54
Pea10	22 ± 0	94 ± 4	200 ± 4	40 ± 1	110 ± 5	313 ± 43
Soy	48 ± 30	322 ± 194	968 ± 379	19 ± 2	208 ± 58	996 ± 359
Wheat	134 ± 8	445 ± 30	1051 ± 203	619 ± 430	1249 ± 500	2120 ± 618

Appendix Tables A1–A2

Data availability

The data are contained within the article; additional information is available upon request.

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Appendix 3

Publication III

Kaleda, A., Sharma, N., **Jakobson, K**., Stulova, I., & Rosenvald, S. (2025). Fermentation by lactic acid bacteria during pea protein isolation reduces undesirable flavors and changes techno-functional properties. Food Chemistry, 492(Part 1), 145380. doi.org/10.1016/j.foodchem.2025.145380

Food Chemistry 492 (2025) 145380

Contents lists available at ScienceDirect

Food Chemistry

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

Fermentation by lactic acid bacteria during pea protein isolation reduces undesirable flavors and changes techno-functional properties

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ARTICLE INFO

Keywords:
Plant protein isolation
Pea protein
Protein fermentation
Lactic acid bacteria
Sensory analysis
Protein techno-functional properties
Isothermal microcalorimetry

ABSTRACT

Plant protein isolates often have undesirable flavors. The conventional protein isolation method involves alkaline solubilization and acidic precipitation. We investigated whether replacing chemical acidification with lactic acid fermentation could improve the sensory properties of pea protein isolate without compromising its techno-functional properties. We prepared two fermented isolates using different starter cultures and one conventional control. Both cultures grew in the protein solution, primarily consuming sucrose naturally present in peas, while producing lactic acid, lowering pH to 4.8 in 4–7 h. The protein yield was unaffected by fermentation, but undesirable flavors were reduced, and bitterness decreased threefold. Powder solubility decreased by 20 %, but water absorption and foaming capacity increased twofold. Oil holding capacity, emulsion activity, protein surface hydrophobicity, and in vitro protein digestibility showed minimal changes. Lactic acid fermentation during protein isolation appears to be a promising approach for improving the sensory properties of protein isolates.

1. Introduction

Plant protein concentrates and isolates are needed for the development of sustainable meat and dairy alternatives, but unlike animal proteins, plant proteins typically have strong, undesirable raw material flavors, including bitterness and astringency (Jakobson et al., 2023; Sarkar, 2024). This limits their application possibilities and lowers consumer acceptance of plant-protein-based products (Vaikma et al., 2022), making the elimination of off-flavors in plant protein ingredients essential.

Off-flavors in plant proteins are caused by different classes of molecules (Leonard et al., 2023; Mittermeier-Kleßinger et al., 2021). Volatile compounds, such as aldehydes, ketones, and alcohols, are commonly responsible for off-odors (Mittermeier-Kleßinger et al., 2021). For example, hexanal and n-hexanol are often associated with grassy and legume-like odors (Vaikma et al., 2021). Bitter taste is imparted by a variety of compounds, including glucosides, glucosinolates, flavonoids, phenols, terpenoids, terpenes, saponins, monoglycerides, fatty acids, and peptides (Vaikma et al., 2022). Astringency, a mechano-sensation in the oral cavity, is partially attributed to the action of phenolic compounds, including tannins, phenolic acids, flavonoids, cyanidins, procyanidins, and proanthocyanidins (Sarkar, 2024). Reducing or modifying these compounds could significantly improve the sensory profile of a plant protein ingredient.

The most effective way to improve the flavor of plant proteins is to mitigate it at the source by selecting or breeding crops with lower concentrations of undesired flavor-active compounds (Trindler et al., 2022). For example, a pea variety without pea flavor has been reported (John Innes Centre, 2023). However, many flavor-active compounds are vital secondary metabolites and facilitate defense mechanisms in plants (Mithöfer & Boland, 2012). Consequently, off-flavor mitigation must be carried out during processing. The flavor of a protein ingredient develops during the various steps involved in protein extraction, as some molecules are eliminated and others are created through enzymatic activity, oxidation, solubilization, thermal treatment, etc. (Trindler et al., 2022). More intensive processing and purification, typically used in the production of high-protein ingredients, can result in a more neutral sensory profile but also damage the protein and affect its techno-functional properties (De Angelis et al., 2024; Hopf et al., 2024). A targeted approach to degrading aldehydes and ketones using purified alcohol dehydrogenases and bacterial suspensions producing these enzymes has been shown to significantly lower the concentration of

This article is part of a Special issue entitled: '3rd NIZO Plant Protein' published in Food Chemistry.

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https://doi.org/10.1016/j.foodchem.2025.145380
Received 27 March 2025; Received in revised form 20 June 2025; Accepted 28 June 2025
Available online 3 July 2025
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aldehydes in pea protein, reducing its pea flavor with minimal alteration of techno-functional properties (Nugroho et al., 2024). Fermentation of plant proteins and plant-based foods has also shown great potential for improving their flavor (Abbaspour, 2024; Zipori et al., 2024).

Fermentation can be applied at various stages in the production of plant protein ingredients: to the raw material before extraction, during protein enrichment, or to the extracted protein. The first and third options are the most frequently reported in the literature and can be applied to flours and protein-rich ingredients produced with a variety of methods, such as milling, dry or wet fractionation. Pei et al. (2022) investigated the fermentation of pea flour by *Lactobacillus rhamnosus*, observing a significant reduction in volatiles associated with unwanted odors, such as nonanal, decanal, octanal, 1-hexanol, and 2-ethyl-1-hexanol. Li et al. (2021) fermented yellow pea flour using five lactic acid bacteria (LAB) strains and found that the resulting volatile compound profile depended on the strain and fermentation time, with *Lactobacillus acidophilus* showing the most potential. Another study examined fermentation of a pea protein isolate (PPI) using six LAB strains and found that bitterness and green, earthy, and pea-like odor attributes were reduced, with the best result achieved by *Lactiplantibacillus plantarum* after 24 h; however, fermentation also significantly decreased techno-functional properties due to additional thermal treatments applied to inactivate the bacteria (García Arteaga et al., 2021). More studies have reported the reduction of plant-like off-flavors and bitterness in fermented pea protein isolates (García Arteaga et al., 2022; Shi et al., 2021). When fermentation is applied to the raw material, it can also affect starch, fibers, and lipids, which may be undesirable (e.g., if starch is a coproduct) or unnecessary, wasting resources on treating a material that contains only around 25 % of protein, as in the case of pea flour (Cousin, 1997; Kumitch et al., 2020; Li et al., 2021). Moreover, both of the aforementioned fermentation options introduce additional steps to the wet protein isolation procedure, significantly increasing production time and costs. Fermentation may also be uneconomical when applied to dry-fractionated protein concentrates, as it requires the addition of water and subsequent its removal, negating the sustainability and resource use advantage of the dry fractionation method. As an alternative, fermentation during the wet protein isolation process has been proposed (Emkani et al., 2021; Emkani et al., 2023; Schaffner & Beuchat, 1986).

The conventional wet protein isolation method starts with alkaline solubilization of protein, followed by gravimetric separation of insoluble fractions and acidification to isoelectric point of protein to precipitate it from the supernatant. Many plant proteins are insoluble at their isoelectric point, commonly in the pH range of 4–5, and their solubility increases when the pH deviates from this value (Etzbach et al., 2024). To adjust the pH during isolation, mineral alkali and acids are added. However, LAB can also produce organic acids during fermentation and decrease the pH to the isoelectric point of plant proteins, potentially eliminating the need for chemical acidification. Two studies by Emkani et al. (2021, 2023) demonstrated the feasibility of this approach. In the first study, pea protein solution was acidified from pH 7.5 to 4.8 using combinations of *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis* (Emkani et al., 2021). They observed the same protein yield as direct acid addition but also reported an increase in protein solubility due to proteolytic activity. In the second study, fermentation using *Streptococcus thermophilus* and *Lactiplantibacillus plantarum* showed degradation of antinutrients like trypsin inhibitors and phytates, which depended on the LAB strain used (Emkani et al., 2023). However, the effect of LAB fermentation during protein isolation on the sensory and techno-functional properties of the isolate has not been reported.

To address this knowledge gap, we investigated the influence of fermentation by different LAB starter cultures on the sensory, physicochemical, and techno-functional properties of a pea protein isolate when fermentation is applied during the conventional pea protein isolation process to acidify and precipitate the protein. We investigated these properties because they are important quality attributes of protein ingredients used in the development of plant-based dairy and meat alternative products.

2. Materials and methods

2.1. Materials

Chemicals and standards used in all analytical measurements were of analytical grade and sourced from Merck KGaA (Darmstadt, Germany), Honeywell (Charlotte, NC, USA), Biosynth Ltd. (Compton, UK), Thermo Fisher Scientific Inc. (Waltham, MA, USA), and Neogen Corporation (Lansing, MI, USA).

Protein was extracted from field pea, variety “Kirke”, grown in Estonia in 2019 (Olle et al., 2020). The material was milled without dehulling and stored at −20 °C. On a dry weight basis (dwb), the flour contained 23.66 ± 0.34 g/100 g dwb of protein and 0.94 ± 0.03 g/100 g dwb of fat. The dry matter was 87.93 %.

Five commercial LAB starter cultures (SC) encoded as SC1–SC5 with different bacterial compositions were selected based on their ability to ferment peas, as specified by the manufacturer. The declared bacterial composition of each starter culture was as follows: SC1 (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subspieces *bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium lactis, Lactiplantibacillus plantarum* or formerly *Lactobacillus plantarum*), SC2 (*L. acidophilus*, *Lactobacillus paracasei*, *Pediococcus pentosaceus*), SC3 (*L. bulgaricus*, *B. lactis*, *Lpb. plantarum*), SC4 (*S. thermophilus*, *L. bulgaricus*), and SC5 (*S. thermophilus*, *L. bulgaricus*, *L. acidophilus*, *B. lactis*). The starter cultures were resuspended in sterile 0.85 % NaCl solution at 100 times the recommended dose and stored at 4 °C for 1 h until use. All stock solutions were standardized and prepared to have the same acidification capacity as defined by the manufacturer.

2.2. Protein solubilization

The pea protein extraction method was based on previously reported methods (Emkani et al., 2023; Gultekin Subasi et al., 2024). To determine the optimal extraction conditions for our material, a small-scale preliminary optimization experiment was conducted, resulting in the optimized method described below. Pea flour was mixed with distilled water at 21 °C in a flour-to-water ratio of 1:6, then the pH was increased to 7.5 using 2 M NaOH. The suspension was stirred for 1 h, and the pH was maintained by adding more NaOH every 5 min. After stirring, the suspension was centrifuged at 3000 ×*g* for 10 min at 21 °C to remove insoluble material. The supernatant was collected and split to prepare three different pea protein isolate (PPI) powders. PPI_Ctrl was prepared through chemical precipitation, while PPI_SC1 and PPI_SC2 were produced through fermentation. The subsequent steps are described in Sections 2.4–2.5.

2.3. Screening of starter cultures

Small-scale preliminary experiments were conducted to study fermentation kinetics of starter cultures and identify the best candidates for the main experiment. Five SCs and one spontaneous control were investigated. A protein solution prepared as described in Section 2.2 was inoculated by adding 1 % of a stock solution and, optionally, supplemented with 0.5 % of sucrose. Fermentation was conducted at 40 °C for 18 h. The acidification activity was measured in a 50 mL volume using a multichannel pH-meter (iCinac, AMS S.r.l., Rome, Italy). Simultaneously, heat generation due to bacterial metabolic activity was monitored in 2 mL sealed vials using a 48-channel isothermal microcalorimeter (TAM IV-48, TA Instruments, New Castle, DE, USA) (Kaleda et al., 2020; Stulova et al., 2013). The experiments were conducted in two biological replicates. Starter cultures for PPI production were selected by evaluating pH and heat flow curves and an informal

sensory assessment.

2.4. Protein precipitation using LAB fermentation

Starters SC1 and SC2 were selected in preliminary experiments detailed in Section 2.3. A protein solution prepared as described in Section 2.2 was poured into 5 L bottles, heated to 40 °C in a water bath, and then inoculated by adding 1 % of a stock solution. The bottles were incubated at 40 °C while being stirred. Once the pH reached 4.8, the suspension was centrifuged at 3000 ×*g* for 10 min at 21 °C to recover the protein paste. This paste was then resuspended in distilled water in a 1:1 ratio, neutralized to pH 7.0 with 2 M NaOH, and then lyophilized. The two collected fermented protein powders were labeled as PPI_SC1 and PPI_SC2.

2.5. Chemical protein precipitation

Isoelectric protein precipitation was achieved by adding 4 M HCl to the protein solution prepared as described in Section 2.2 until the pH reached 4.8. The protein was then collected by centrifugation (3000 x*g*, 10 min, 21 °C), resuspended in distilled water at a 1:1 ratio, neutralized with 2 M NaOH to pH 7.0, and then lyophilized. The collected protein powder was used as a control (PPI_Ctrl).

2.6. Sugars and organic acids content during fermentation

Liquid samples collected during fermentation with SC1 and SC2 in the main experiment were vortexed for 30 s, then centrifuged at 14,000 ×*g* for 10 min at 21 °C. The supernatant was filtered through a pre-washed 3 kDa molecular weight cut-off filter (Amicon® Ultra-0.5, Merck KGaA, Germany). Sugars and organic acids were quantified using a high-performance liquid chromatography (HPLC) system (Alliance 2695 system, Waters Corp., Milford, MA, USA), equipped either with an Aminex HPX-87P column for sugars or an Aminex HPX-87H column for organic acids (7.8 × 300 mm, 9 µm particle size, Bio-Rad Laboratories, Inc., CA, USA). For sugar analysis, a BioRad Micro-Guard Cation P guard column (4.6 × 30 mm, 9 µm particle size) was used with isocratic elution of ultrapure water at a flow rate of 0.6 mL min^{-1} at 85 °C. H guard column (4.6 × 30 mm, 9 µm particle size) with isocratic elution of 5 mM H_2SO_4 at a flow rate of 0.6 mL min^{-1} at 35 °C was used for organic acid analysis. A Waters 2414 refractive index detector was used for the detection and quantification of substances, paired with a Waters 2487 Dual Absorbance Detector for organic acid analysis. Calibration curves for quantification were made by injecting pure standards at known concentrations.

2.7. Volatile compounds in PPIs and raw flour

Extraction of volatiles from PPI_SC1, PPI_SC2, PPI_Ctrl, and the initial raw flour was carried out using solid phase microextraction. A 60 mg sample was weighed into a 10 mL vial containing a magnetic stirrer. 940 µL of Milli-Q water was added to the vial to make a 6 % solution. A solid phase microextraction fiber (30/50 µm DVB/Car/PDMS Stableflex, length 1 cm) was used to adsorb/absorb volatile compounds from the headspace for 40 min at 50 °C. The volatiles were then desorbed into a gas chromatography injection port for 5 min. Identification and semi-quantification of volatile compounds was performed using a gas chromatograph system (2030; Shimadzu, Kyoto, Japan) equipped with a mass spectrometer (8050NX Triple Quadrupole; Shimadzu, Kyoto, Japan). A ZB5-MS column (30 m length × 0.25 mm i.d. × 1.0 µm film thickness; Phenomenex, Torrance, CA, USA) was used with helium as the carrier gas at a linear velocity of 35 cm s^{-1}. The oven was programmed to ramp up from 40 °C at a rate of 5 °C min^{-1} to a temperature of 190 °C, and then from 190 °C to 280 °C at a rate of 25 °C min^{-1}, with an additional holding time of 3 min, resulting in a total run time of 36 min. Mass spectra were obtained at an ionization energy of 70 eV with a mass-to-charge ratio scan range of 35 to 300. Non-targeted identification of volatile compounds was carried out using GCMS solution software (Shimadzu, Japan) and retention indices. Experimental retention indices were calculated using the retention times of the eluting compounds, normalized to the retention times of adjacent n-alkanes. The identification of the compounds was verified by comparing experimental retention indices to the NIST17 and FFNSC libraries. Semi-quantitative approach against an internal standard (4-methyl-2-pentanol; 333 ppb) was used to quantify identified volatile compounds.

2.8. Sensory assessment of PPIs

The quantitative descriptive sensory analysis of PPI_SC1, PPI_SC2, and PPI_Ctrl was conducted by 8 assessors (average age 33 ± 8), who had previous experience in evaluating plant protein isolate powders (Jakobson et al., 2023). Prior to the main assessment sessions, a preliminary session was conducted with selected samples to familiarize the assessors with the samples and to refine the assessment method. The final assessment method was based on relevant scientific sources, in-house protocols, and discussions with the sensory panel. The samples were prepared as 6 % water dispersions in filtered drinking water and served at 21 °C in transparent sniffing glasses with cover slips. The evaluation was performed independently by the assessors. Each sample was coded with a random three-digit code, and the order of assessment was randomized for each assessor according to the Williams' Latin Square design. The evaluation took place in a dedicated sensory room where external factors that could interfere with the evaluation were eliminated, in accordance with ISO 8589:2007 (ISO, 2007). The samples were assessed in two replicates in one session. The evaluation on a 10-point scale began with odor modality, followed by flavor and texture. The attributes are defined in Table 1. A commercial pea protein isolate was used as a reference to help the assessors understand the scale better. Data collection was carried out using RedJade sensory software, version 6.1.1 (RedJade Sensory Solutions LLC, Martinez, CA, USA).

Table 1
Sensory attributes and their definitions.

Attribute	Definition and scale
Odor	0—"none", 1—"very weak", 5—"moderate", 9—"very strong".
Overall intensity	Overall odor intensity.
Pea	Odor associated with raw legumes.
Sour	Perceivable sourness in odor associated with vinegary, yoghurt-like, fermented, citric, etc.
Sweet	Perceivable sweetness associated with sugar, caramel, toffee, etc.
Off-odor intensity	Non-characteristic odors like aged, chemical, dusty, rancid, metallic, animal, earthy, papery, etc.
Flavor	0—"none", 1—"very weak", 5—"moderate", 9—"very strong".
Overall intensity	Overall flavor intensity.
Pea	Taste associated with raw legumes.
Sour	Perceivable sourness in taste associated with vinegary, yoghurt-like, fermented, citric, etc.
Sweet	Perceivable sweetness in taste associated with sugar, caramel, toffee, etc.
Salty	Perceivable saltiness associated with table salt and minerals.
Bitter	Perceivable bitterness associated with caffeine, quinine, etc.
Astringent	Perceivable astringency—dry, sharp, mouth puckering.
Off-taste intensity	Non-characteristic taste like aged, chemical, dusty, rancid, metallic, animal, earthy, papery, etc.
Aftertaste intensity	Perceivable aftertaste intensity 5 s after swallowing.
Texture	
Graininess (size)	Size of perceivable grains in the sample. 0—"none", 1—"very small", 5—"moderate", 9—"very big".
Graininess (amount)	Amount of perceivable grains in the sample. 0—"no particles", 1—"few particles", 3—"some particles", 5—"several particles", 7—"many particles", 9—"mostly particles".

2.9. Physicochemical, techno-functional, and digestibility properties of PPIs

The total protein content was determined by the Kjeldahl method (N × 6.25).

The following analyzes were performed for PPI_SC1, PPI_SC2, and PPI_Ctrl.

For color measurement, the PPI powders were placed in 8 cm × 8 cm transparent plastic mini-grip bags, forming a 5-mm-thick layer, and the color was measured at three different spots using a portable spectrophotometer (NS810, 3nh Shenzhen Threenh Technology Co., Ltd., Shenzhen, China) set to measure in the CIELAB color space using illuminant D65 and 2°.

Water holding capacity (WHC), oil holding capacity (OHC), water solubility index (WSI), foaming capacity (FC), foaming stability (FS), emulsification activity (EA), and emulsification stability (ES) of PPI powders were measured as reported previously without modifications (Jakobson et al., 2023).

In vitro protein digestibility (IVPD) of PPI powders was determined as described by Hsu et al. (1977) and Espinosa-Ramírez et al. (2018) with some modifications. Briefly, 10 mL of protein dispersion containing 6.25 mg of protein mL^{-1} was maintained at 37 °C in a water bath. The pH was adjusted to 8.00, by using 0.1 N NaOH or HCl during stirring. An enzyme solution prepared with trypsin (>6000 BAEE units mg^{-1}) at a concentration of 1.6 mg mL^{-1} was maintained on ice until use. Then, 1 mL of the enzyme solution was added to the protein suspension, and the pH drop was recorded after 10 min. The IVPD was calculated using the equation $IVPD, \% = 210.464 - 18.1x$, where x is the pH value after 10 min.

Protein surface hydrophobicity of PPI powders was evaluated using the method developed by Cao et al. (2016) with some modifications (Sharma et al., 2023). The method is based on measuring the formation of a complex between the basic and aromatic amino acid residues and the anionic form of Coomassie Brilliant Blue G-250 (CBBG) stain due to hydrophobic interactions. This method is suitable for partially soluble protein powders. A sample dispersion (1.2 mL) with 5 mg mL^{-1} of total protein content was prepared using 20 mM phosphate buffer at pH 6.0. Then, 300 μL of a 0.1 mg mL^{-1} CBBG solution in Milli-Q water was added. A sample blank was prepared as described above, but without adding the CBBG solution. A reagent blank without protein was prepared by combining 1.2 mL of the phosphate buffer with 300 μL of the CCBG solution. The dispersions were agitated in a multi-shaker (Thermo Mixer C, Eppendorf, Connecticut, United States) at 2000 rpm for 4 min at 21 °C and then centrifuged at 2000 ×*g* for 10 min at 4 °C. The supernatant was transferred to another tube without disturbing the pellet using a pipette and centrifuged again under the same conditions. Finally, the supernatant absorbance at 585 nm was measured using a UV-VIS spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), which was zeroed with the phosphate buffer. The result was expressed as $bound\ CBBG, \mu g = (Reagent\ blank\ absorbance - Sample\ abs. + Sample\ blank\ abs.)/(Reagent\ blank\ abs.) \times 30\ \mu g$.

2.10. Statistical analysis

Starter culture screening tests were performed in biological duplicates. Protein content, color, IVPD, WHC, OHC, WSI, FC and FS, EA and ES, surface hydrophobicity, and volatile compounds were measured in analytical triplicates. Sugars, organic acids, and sensory profile were analyzed in analytical duplicates. Statistical analysis and data visualization were performed using R version 4.3.0 (The R Foundation for Statistical Computing, Vienna, Austria). Kruskal-Wallis tests followed by Conover-Iman post hoc tests with Bonferroni adjustments were performed using R package "conover.test" version 1.1.5. The significance level was set at 0.05.

3. Results and discussion

3.1. Starter culture selection

Initially, we selected five commercial starter cultures containing different combinations of LAB, which were previously reported to grow in legume protein preparations. For instance, successful fermentation has been achieved using *Lpb. plantarum* (Emkani et al., 2023; Shi et al., 2021; Skalickova et al., 2022), *L. acidophilus* (Demarinis et al., 2022; Parra et al., 2013; Schaffner & Beuchat, 1986), *L. paracasei* (Demarinis et al., 2022), *L. lactis* (Zipori et al., 2024), *S. thermophilus* (Emkani et al., 2023; Schaffner & Beuchat, 1986; Zipori et al., 2024), and *P. pentosaceus* (Zipori et al., 2024). Moreover, some co-cultures exhibited a synergistic effect, accelerating fermentation and improving properties further (Schaffner & Beuchat, 1986; Yang et al., 2023). Different from traditional fermentation applied to protein-based foods such as tofu, yoghurt, or cheese, for the purpose of protein isolation, proteolytic activity was considered undesirable. To narrow our selection, we conducted a preliminary screening experiment. We also investigated whether the acidification activity of these bacteria could be enhanced through sugar supplementation (Kaleda et al., 2020; Youssef et al., 2020).

Fig. 1 presents the average fermentation profile of five selected starter cultures (SCs) in comparison to a spontaneous control. Isothermal microcalorimetry heat flow curves in Fig. 1a and pH curves in Fig. 1b show that all starter cultures were capable of growth and acidification of the pea protein solution to a pH of 4.8, necessary for the precipitation of pea globulins (Emkani et al., 2023). Despite protein solubilization at pH 7.5, the initial fermentation pH was around 7.0, resulting from the separation of solids and the subsequent heating of the protein solution to 40 °C.

The fastest acidifier was SC1, followed by SC4 and SC5, achieving pH 4.8 in 3.7 h, 4.6 h, and 4.6 h, respectively (Fig. 1c). These SCs exhibited high and singular heat flow peaks, beginning at around 1.5 h and ending by 6 h, in contrast to SC2 and SC3, which produced lower heat flows that peaked later but maintained heat production for longer, finishing at around 13 h. *S. thermophilus* was the only species present in SC1, SC4, and SC5, and absent in SC2 and SC3. *S. thermophilus* are known to be fast acidifiers in dairy milk, but their performance widely varies between strains (Beux et al., 2020). The heat flows of SC1–SC5 peaked and metabolic activity began to weaken as pH dropped below 5 (Fig. 1a, b), which is outside the optimum pH range for LAB (Hofvendahl & Hahn Hägerdal, 2000). The acidification speed of all SCs sharply decreased after heat flow peaked, but pH continued to slowly decrease until the end of the experiment, with the final pH ranging from 3.7 to 4.3, as shown in Fig. 1d. Notably, the slowest acidifier, SC3, achieved the lowest final pH of 3.7, which is the lowest growth boundary for *Lpb. plantarum* (Giraud et al., 1991). However, we acknowledge that the observed acidification and heat production profiles may not generalize to the species level, as strain variability can be high, making the comparison with literature difficult. The differences should be attributed rather to the properties of each strain in the starter culture consortium, such as the presence of specific enzymes involved in sugar utilization. In addition, the dose of each starter culture was calculated to have the same acidification performance in some other substrate, as specified by the manufacturer, and not by the number of viable cells, which may also partially explain the differences in the acidification profiles.

As evident from Fig. 1a, all SCs had completed fermentation and entered the deceleration phase before the onset of the exponential growth phase in the spontaneous control. Furthermore, Fig. 1b shows that even the slowest acidifier, SC3, reached a pH of 4.8 before the pH of the spontaneous control began to decrease. This result is important, indicating that the initial protein solution had a low microbiological load and that costly and protein-denaturing pasteurization before

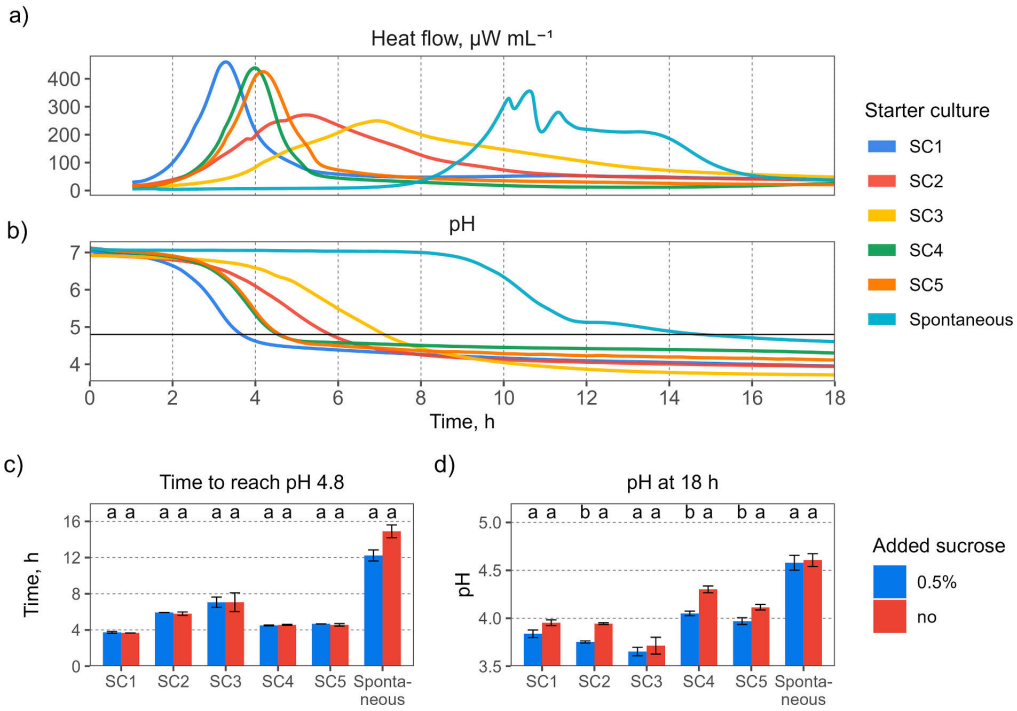

Fig. 1. Average (biological $n = 2$) fermentation profile of different starter cultures (SC) at 40 °C compared to a non-inoculated spontaneous control. a) Heat flow without sucrose supplementation measured by isothermal microcalorimetry, b) pH profile without sucrose supplementation, the black horizontal line shows pH 4.8, c) time taken to reach pH 4.8, d) pH at 18 h point.

fermentation may not be necessary, as SCs can outgrow and suppress spoilage bacteria (Ibrahim et al., 2021). The multiple peaks detected in the heat flow of the spontaneous sample and in SC2 may be caused by metabolic switching following changes in nutrient and oxygen availability (Kattel et al., 2024; Stulova et al., 2013; Zaharia et al., 2013).

As shown in Fig. 1c, there was no statistically significant difference in the acidification time with sucrose supplementation ($p > 0.05$), except potentially for the spontaneous control sample ($p = 0.058$). However, the added sucrose prolonged the duration of acidification activity, resulting in a final pH 0.06–0.25 pH units lower at the 18-h mark in all SCs (Fig. 1d), though the difference was statistically significant only for SC2, SC4, and SC5 ($p < 0.05$). This indicates that the initial pea flour contained a substantial amount of soluble saccharides, enabling the SCs to acidify to pH 4.8. This is a major advantage of conducting fermentation during the protein isolation process as opposed to after isolation, as it allows for the utilization of native sugars that would otherwise be a waste stream. Published studies that fermented proteins after their isolation had to add sugars to assist bacterial growth (García Arteaga et al., 2021; Kaleda et al., 2020; Zipori et al., 2024) because commercial plant protein isolates typically contain only around 2 % of carbohydrates (Jakobson et al., 2023).

After 18 h of fermentation, we conducted an informal sensory assessment and noticed that in comparison to the spontaneous sample, SC1, SC5, and particularly SC2 and SC3 exhibited a weaker pea-like odor. Furthermore, SC1, SC2, and especially SC3 showed a marked decrease in pea flavor. Although SC3 demonstrated the strongest flavor improvement, for further study we selected the second best—SC2, since it was a faster acidifier. Additionally, we selected SC1, which provided modest sensory improvement, but had the shortest acidification time overall.

3.2. Fermentation influence on the protein isolation process

After selecting starter cultures and conditions in screening experiments, we prepared three pea protein isolates (PPI). Two PPIs were fermented at 40 °C using SC1 and SC2, labeled as PPI_SC1 and PPI_SC2, respectively. The third was acidified chemically (PPI_Ctrl) at 21 °C, emulating a typical protein isolation procedure.

As shown in Table 2, the acidification time of PPI_SC1, 3.9 h, was similar to the preliminary test, but PPI_SC2 required 7 h instead of the expected 6 h to reach pH 4.8. This discrepancy can be attributed to the 80-fold larger volume and some variations in solution preparation and environmental conditions, including weighing and dosing accuracy, mixing accuracy and intensity, timing, temperature control precision and stability, and oxygen availability. In contrast, chemical acidification of PPI_Ctrl took only a few minutes. Considering this, an acid can be added at the end of fermentation to complete a slow process or at the beginning to adjust the protein solution to a neutral pH suitable for LAB, if a high pH was applied for protein solubilization. This approach may also allow the use of non-acidifying bacteria (Nugroho et al., 2024), but it loses the advantage of eliminating one chemical from the process entirely.

Table 2
Protein content and yield of pea protein isolates (PPI) precipitated using a starter culture (SC) or chemically (Ctrl). Dwb—dry weight basis.

	PPI_SC1	PPI_SC2	PPI_Ctrl
Time to reach pH 4.8, h	3.9	7.0	0.1
Mass yield, % dwb	17.7	17.8	17.9
Protein content, % dwb	84.2	84.6	83.6
Protein recovery, % dwb	63.2	63.7	63.1

The raw pea flour contained 23.6 % of protein on a dry weight basis (dwb), which falls near the lower end of the 22.6–34.8 % range reported for locally grown field pea varieties (Olle et al., 2020). Due to this, and also mild protein solubilization conditions, the mass yield of PPI from the initial flour was relatively modest, around 18 % dwb, as shown in Table 2. Yet, this yield was higher than reported in a study by Manouel et al. (2024), in which the yield was 14.1–16.3 % dwb and depended on pea growth environment and seed protein content. In our study, the yield of fermented isolates was 0.1–0.2 % lower than that of PPI_Ctrl, a difference that is not practically significant, and thus the mass yield of all PPI samples can be considered equal. A similar study also found no difference in yield between fermented and chemically precipitated PPIs (Emkani et al., 2021).

The protein content of PPIs was approximately 84 % dwb, a value close to 83 % reported by Emkani et al. (2023) for fermentation-precipitated PPI. Typically, PPI protein content ranges between 75 % and 89 %, depending on the raw material and extraction parameters (Allotey et al., 2022; Kornet et al., 2022; Manouel et al., 2024). The protein content of fermented PPIs in our study was 0.6–1 % higher than in PPI_Ctrl. Given the slightly lower mass yield of fermented PPIs and the 0.1–0.6 % higher protein recovery compared to PPI_Ctrl, a combination of two effects may have contributed to this small and practically insignificant difference. The bacteria likely degraded and consumed some residual carbohydrates, increasing the protein concentration in PPI_SC1 and PPI_SC2. At the same time, higher temperature during long fermentative acidification may have denatured and aggregated some proteins, decreasing their solubility (Kinsella & Melachouris, 1976) and allowing for more protein to precipitate and be collected. Our protein recovery of 63 % was higher than the 54–61 % reported in the literature for pea (Allotey et al., 2022; Hansen et al., 2022; Kornet et al., 2022; Tian et al., 1999). Optimization of extraction conditions, such as higher pH and larger water volume, can improve protein solubilization (Gultekin Subasi et al., 2024; Hansen et al., 2022), and correspondingly, PPI mass yield and protein recovery. However, we selected mild extraction conditions with lower resource use. They are more suitable for large-scale production, preserve protein techno-functional properties (Hansen et al., 2022), and the neutral initial pH of the protein solution is preferred by LAB, which have optimal lactic acid production in the pH range of 5–7 (Hofvendahl & Hahn Hägerdal, 2000).

3.3. Sugars and organic acids

Pea seeds contain 7–18 % of soluble sugars, with concentrations varying among pea varieties (Cousin, 1997). The most abundant sugars are sucrose and its derivatives—raffinose, stachyose, and verbascose, also known as raffinose family oligosaccharides (RFOs). Typically, sucrose, stachyose, and verbascose concentrations are roughly comparable, but raffinose concentration is 2–3 times lower (Cousin, 1997; Fenn et al., 2022). The metabolism of these sugars involves different enzymatic pathways. *Lactobacilli* species can hydrolyze sucrose extracellularly using invertases or sucrases, or modify it during transport into the cell and then hydrolyze (Gänzle & Follador, 2012). Some *Lactobacilli* can express enzymes necessary for RFO metabolism, such as the key enzyme α-galactosidase, but multiple pathways for sucrose utilization indicate that it is a preferred substrate (Gänzle & Follador, 2012).

Fig. 2a shows the soluble sugars that were found in the protein solution before and during fermentation. Initially, oligosaccharides were the most abundant, followed by disaccharides sucrose and maltose, and their monosaccharides glucose, fructose, and galactose. Due to the chromatographic analysis not sufficiently resolving oligosaccharides,

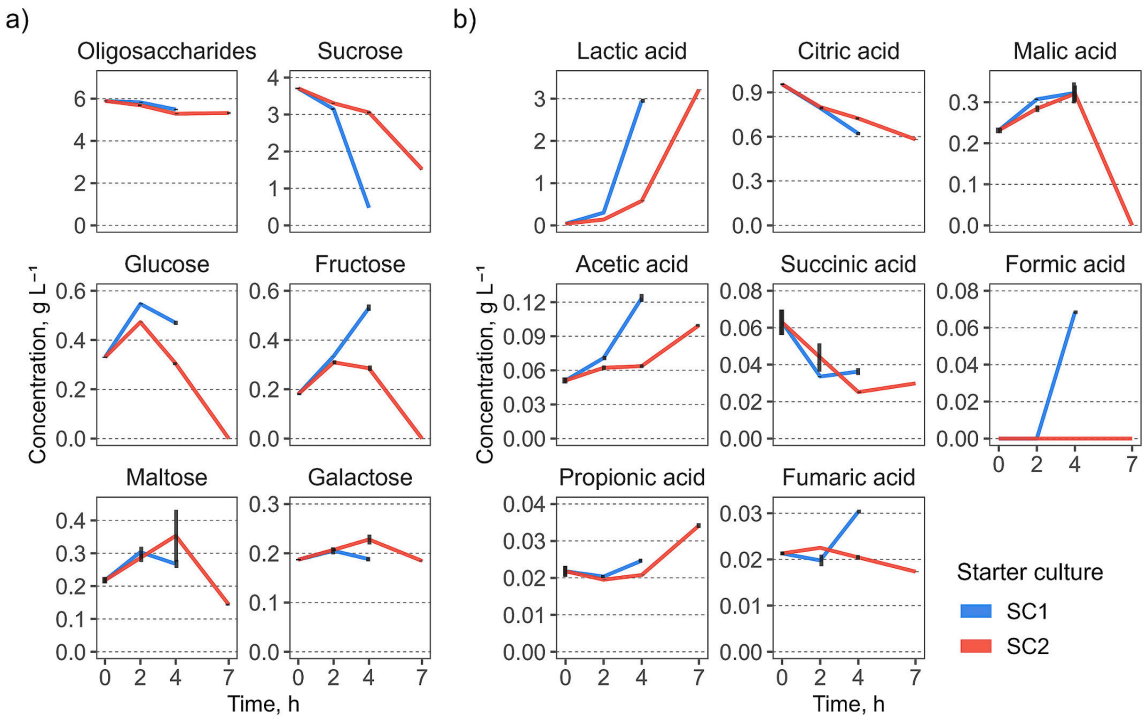

Fig. 2. Average concentrations in g L^{-1} of a) sugars and b) organic acids detected in the protein supernatant during fermentation. Vertical lines show standard deviations (analytical $n = 2$).

the concentration of RFOs was estimated as total oligosaccharides. The data show that sucrose was utilized by both SCs. Initially, the protein solution contained 3.7 g L^{-1} of sucrose. SC1 rapidly decreased its concentration to 0.5 g L^{-1} at 4 h point. In contrast, SC2 decreased sucrose more slowly and after 7 h its concentration was 1.5 g L^{-1}. As sucrose was hydrolyzed, glucose and fructose were released, and their concentration initially increased. By the end of fermentation SC2 completely depleted both glucose and fructose, but in the SC1 solution their concentration was 0.5 g L^{-1}. The initial solution also contained 0.2 g L^{-1} of maltose. During fermentation, its concentration initially slightly increased for both SCs but then decreased. Since maltose is usually a product of starch hydrolysis (Gänzle & Follador, 2012), it may have been produced from amylose traces remaining in the solution. Both SCs decreased the concentration of oligosaccharides from 5.9 g L^{-1} by only around 10 %, and the concentration of galactose, a product of RFO hydrolysis, remained relatively unchanged until the end of fermentation. Previous studies have shown that LAB species and their co-cultures can metabolize RFOs, but efficiency strongly depends on the specific bacterial strains and their co-culture interactions, substrate properties, and fermentation conditions (Donkor et al., 2007; Gandhi, 2021; Kahala et al., 2023; Verni et al., 2019). For instance, similarly to our results, Emkani et al. (2023) found that while monocultures of *S. thermophilus* and *Lpb. plantarum* decreased RFOs in a pea protein solution substantially, in co-culture their RFO consumption was only around 10 %. Regardless, the total concentration of sugars in the initial pea protein solution was sufficient for fermentation, and they were not completely depleted by the time pH reached 4.8, explaining why sucrose supplementation did not shorten the acidification time.

LAB consume sugars and produce organic acids, thereby reducing the pH. Fig. 2b shows the organic acids detected in the initial protein solution and during fermentation. At the beginning, citric acid was the dominant acid at 0.95 g L^{-1}, while the concentrations of lactic, malic, acetic, succinic, propionic, and fumaric acids were in the range of 0.02–0.23 g L^{-1}. Mabesa et al. (1979) reported that 100 g of raw peas contained 0.067 g of citric acid, 0.016 g of malic acid, 0.007 g of lactic acid, and 0.002 g of succinic acid. After accounting for dilution, these concentrations were an order of magnitude lower than ours. It is possible that more acid was produced in the steps prior to and during protein solubilization. We also checked for other acids, including gluconic, glucuronic, galacturonic, butyric, isobutyric, valeric, isovaleric, but none were detected. Lactic acid was the primary metabolic product of both SCs, reaching approximately 3 g L^{-1} by the end of fermentation. For comparison, García Arteaga et al. (2021) reported that after 24 h of PPI fermentation by *Lpb. plantarum* and *P. pentosaceus*, lactic acid concentrations reached 3.3 g L^{-1} and 2.4 g L^{-1}, respectively. Both SC1 and SC2 produced other acids, albeit in small quantities. The concentrations of malic, acetic, propionic, fumaric, and formic acids remained in the range of 0.02–0.32 g L^{-1}. Differences in organic acid profiles were observed between SC1 and SC2. SC1 increased the total concentration of organic acids more quickly, reaching 4.2 g L^{-1} after 4 h, whereas SC2 reached 4.0 g L^{-1} by 7 h. SC1 was the sole producer of formic acid. Differently from the other acids, citric and succinic acids were partially consumed by both SCs, and malic acid was depleted by SC2 after an initial increase. LAB can utilize certain carboxylic acids as a carbon source, although their consumption varies between strains and is impacted by environmental factors, such as the availability of preferred sugars (Radler & Bröhl, 1984). For instance, certain *S. thermophilus* could not utilize malic and succinic acids due to the lack of enzymes in the reductive pathway of the TCA cycle (Kütt et al., 2023). Some *Lpb. plantarum* and L. *paracasei* species can have genetic sequences encoding enzymes needed for citric acid degradation (Kütt et al., 2023). The reduction of malic acid by SC2 at the end of fermentation may be due to malolactic fermentation, in which malic acid is converted to lactic acid, a common process among many *Lactobacillus* and *Pediococcus* species (Radler & Bröhl, 1984).

3.4. Volatile compounds

A total of 79 volatile compounds were detected and semi-quantified in protein isolates and initial pea flour, as listed in Table 3. These volatiles were categorized into 11 molecular groups, with the total concentration by group presented in Fig. 3. The total content of different volatile groups in both fermented PPIs was comparable, but PPI_SC2 had significantly higher concentrations of alcohols and acids. Aldehydes were the most dominant group of volatiles, both in terms of the number of compounds and their total concentration, which is typical for pea proteins (Liu et al., 2023; Shi et al., 2021). The lowest concentration of aldehydes was found in pea flour, while the highest was in the non-fermented isolate PPI_Ctrl. Aldehydes are produced from fatty acids through enzymatic and non-enzymatic pathways and are one of the major contributors to the “green” and “pea-like” off-flavors (Liu et al., 2023; Zhogoleva et al., 2023). Aldehydes have low water solubility and can bind to proteins through hydrogen bonding, hydrophobic interactions, or covalent bonding, which occurs when high concentrations of lysine, arginine, methionine, or cysteine are present (Vatansever et al., 2024). In contrast, alcohols and ketones tend to bind proteins mostly through weak hydrogen bonding (Vatansever et al., 2024). Consequently, aldehydes may tend to concentrate in the protein fraction during protein isolation and have a greater impact on sensory properties.

In comparison to PPI_Ctrl, fermentation considerably reduced the concentration of some aldehydes that were also present in the initial pea flour, including hexanal (grass odor) and nonanal (Table 3). At the same time, fermentation produced other aldehydes like benzaldehyde (almond odor), 2-methylbutanal and 3-methylbutanal (cocoa, chocolate odor), and acetaldehyde (fruity odor), decreasing the overall difference between the total concentrations of aldehydes (Fig. 3). Nevertheless, the total concentration of aldehydes in PPI_SC2 and PPI_SC1 was 12–20 % lower than in PPI_Ctrl. *Lpb. plantarum*, present in SC1, is known to be effective in improving plant material flavor by breaking down volatile compounds causing beany and grassy flavors. *Lpb. plantarum* possesses alcohol dehydrogenase and aldehyde dehydrogenase enzymes that convert aldehydes and ketones into less odorous alcohols or carboxylic acids (Fischer et al., 2022). Shi et al. (2021) fermented pea protein isolates and found that *Lpb. plantarum* significantly reduced aldehyde and ketone content, decreasing their concentration by 42 % and 64 % after 10 h, resulting in a noticeable reduction of unpleasant beany flavors. However, further fermentation led to a large increase of ketones instead. Another study also reported a reduction of undesirable flavors when aldehydes and ketones were hydrolyzed by alcohol dehydrogenase, which was especially effective in the case of heterofermentative LAB strains (Nugroho et al., 2024).

Ketones were the second-largest group of volatiles, and they were mainly generated during fermentation, increasing their total concentration 4-fold. They might play a role in influencing the sensory properties of fermented PPIs, as most possess sweet or earthy odor notes. In contrast, the total content of alcohols decreased during protein isolation, but fermentation increased it, with 1-hexanol and 2-ethyl-1-hexanol being the dominant alcohols. Hexanol is metabolized from hexanal, and this has a positive effect in reduction of “green” odors. A study by Shi et al. (2021) also found that alcohols increased with fermentation time. Both fermented PPIs had higher total concentrations of acids than PPI_Ctrl, with PPI_SC2 containing three times more acids than PPI_SC1. The most dominant acids were acetic and hexanoic. Fermentation also increased esters and sulfur compounds but decreased terpenes and 2-Isobutyl-3-methoxypyrazine.

Overall, fermentation significantly influenced the profile of volatile compounds, with some volatile groups decreasing in concentration and others increasing, but the total concentration of volatiles was 9 % higher in PPI_SC1 and 25 % higher in PPI_SC2 than in PPI_Ctrl. However, the complex nature of human perception of odor-active compounds necessitates an assessment of the impact of these changes using a sensory

Table 3
Volatile compounds detected in pea protein isolates (PPI) precipitated using a starter culture (SC) or chemically (Ctrl) in comparison to the initial pea flour. Concentrations are shown as mean and standard deviation ($n = 3$) in ppb internal standard equivalent. Values in a row not sharing a letter are statistically significantly different. RI exp—experimental retention index.

Volatile compound	Odor description	RI exp.	Pea flour	PPI_Ctrl	PPI_SC1	PPI_SC2
Acids:						
Acetic acid	Vinegar	629	2 ± 0d	4 ± 0c	12 ± 2b	42 ± 4a
Butanoic acid, 2-methyl-	Sweat, cheesy	850	0 ± 0b	0.6 ± 0.1a	0 ± 0b	0 ± 0b
Butanoic acid, 3-methyl-	Sour, stinky feet, sweaty, cheese	844	0 ± 0d	4.2 ± 0.3a	1.3 ± 0.2c	1.9 ± 0.2b
Hexanoic acid	Goat cheese, sweaty	976	1.9 ± 0.3c	2.3 ± 0.1bc	3.4 ± 0.6ab	3.6 ± 0.5a
Alcohols:						
1-Butanol, 2-methyl	Sweet, apricot	739	0 ± 0d	3.5 ± 0.1a	1.8 ± 0.1c	2.5 ± 0.3b
1-Butanol, 3-methyl-	Fusel, alcoholic, whiskey, fruity	736	1.8 ± 0.0c	0.7 ± 0.1d	2.5 ± 0.2b	3.9 ± 0.2a
1-Dodecanol	Earthy, soapy, waxy, fatty	1393	0.5 ± 0.1d	1.3 ± 0.1b	0.8 ± 0.1c	2.3 ± 0.3a
1-Hexanol	Ethereal, fusel, oily, fruity	875	124 ± 25a	15 ± 1c	44 ± 2b	125 ± 5a
1-Hexanol, 2-ethyl-	Citrus, fresh, floral, oily	1034	10 ± 2c	31 ± 0b	44 ± 4a	41 ± 4a
1-Nonanol	Fresh, clean, fatty, floral	1178	7.3 ± 1.1b	2.8 ± 0.3c	10.2 ± 0.0a	9.6 ± 1.0ab
1-Octanol	Waxy, green, orange, aldehydic	1077	8.3 ± 0.7d	14.0 ± 0.8a	10.9 ± 0.0b	9.0 ± 0.1c
1-Octen-3-ol	Mushroom, earthy, green, oily	985	5 ± 1d	20 ± 1c	24 ± 1b	36 ± 2a
1-Penten-3-ol	Ethereal, horseradish, green, radish, chrysanthemum	684	15.5 ± 0.6bc	18.8 ± 1.1a	14.7 ± 0.3c	18.0 ± 1.0ab
1-Tetradecanol	Fruity, waxy, orris, coconut	1670	0 ± 0c	0.2 ± 0.0a	0.1 ± 0.0b	0.2 ± 0.0a
3-Hexen-1-ol, (*E*)-	Green, leafy	869	2.4 ± 0.1a	0 ± 0c	0 ± 0c	0.2 ± 0.0b
Benzyl alcohol	Floral, sweet, almond	1051	0 ± 0b	0 ± 0b	0 ± 0b	1.4 ± 0.1a
Ethanol	Alcohol	479	14.8 ± 0.5a	9.6 ± 1.1b	16.1 ± 1.8a	8.8 ± 0.3b
Aldehydes:						
2-Butenal, 3-methyl-	Sweet, fruity, almond, cherry	795	0 ± 0d	0.8 ± 0.1c	4.5 ± 0.3a	1.5 ± 0.1b
2-Heptenal, (Z)-	Fat, green, mushroom, soap	966	0 ± 0c	2.4 ± 0.3b	2.2 ± 0.1b	4.7 ± 0.4a
2-Hexenal, (E)-	Green, banana, aldehydic, fatty	861	0 ± 0c	36 ± 3a	14 ± 2b	15 ± 1b
2-Nonenal, (E)-	Fatty, green, cucumber, aldehydic	1169	0 ± 0d	2.4 ± 0.2a	0.6 ± 0.0c	1.3 ± 0.1b
Acetaldehyde	Pungent, ethereal, aldehydic, fruity	455	5 ± 1c	55 ± 2b	68 ± 13b	101 ± 8a
Benzaldehyde	Almond	977	0 ± 0d	20 ± 2c	83 ± 0a	45 ± 2b
Benzeneacetaldehyde	Green, sweet, floral, hyacinth	1059	0 ± 0d	3.1 ± 0.1c	9.5 ± 0.0a	7.5 ± 0.3b
Butanal, 2-methyl-	Musty, cocoa, phenolic, coffee	662	2 ± 0d	35 ± 3c	65 ± 1a	47 ± 1b
Butanal, 3-methyl-	Ethereal, aldehydic, chocolate, peach	653	1 ± 0d	14 ± 2c	30 ± 1a	26 ± 1b
Decanal	Fat, orange, stewed	1212	1.5 ± 0.1d	3.7 ± 0.2a	2.3 ± 0.1c	2.7 ± 0.1b
Dodecanal	Soapy, waxy, citrus	1416	0 ± 0b	0.6 ± 0.0a	0 ± 0b	0 ± 0b
Heptanal	Fresh, aldehydic, fatty, green	907	0.8 ± 0.1d	7.8 ± 0.7a	1.5 ± 0.0c	2.5 ± 0.2b
Hexanal	Grass, green	805	68 ± 3d	285 ± 18a	103 ± 11c	171 ± 11b
Hexanal, 2-ethyl-	Fruity, sweet	959	1.7 ± 0.2c	5.7 ± 0.2ab	5.9 ± 0.5a	5.1 ± 0.3b
Nonanal	Waxy, fatty, citrus	1110	13 ± 0d	52 ± 4a	17 ± 1c	24 ± 1b
Octanal	Citrus, fat	1009	0.5 ± 0.0c	7.2 ± 0.7a	0.2 ± 0.0d	0.8 ± 0.1b
Pentadecanal-	Fresh, waxy	1727	1.0 ± 0.1b	10.0 ± 0.8a	0.9 ± 0.1b	7.6 ± 1.2a
Propanal, 2-methyl-	Pungent, green	556	0.3 ± 0.0d	9.7 ± 1.0c	22.7 ± 0.3a	15.1 ± 0.4b
Tetradecanal	Fatty, waxy, amber, incense	1624	0.3 ± 0.0c	10.4 ± 0.8a	0.1 ± 0.0d	1.8 ± 0.3b
Tridecanal	Fresh, clean, aldehydic, soapy	1523	0.4 ± 0.1c	3.4 ± 0.3a	0 ± 0d	0.8 ± 0.0b
Undecanal	Waxy, soapy, floral	1315	0 ± 0b	1.1 ± 0.1a	0 ± 0b	0 ± 0b
Undecanal, 2-methyl-	Fresh, amber, citrus, tuberose, metallic, waxy, coumarinic	1298	0 ± 0c	0 ± 0c	22.3 ± 0.9a	16.8 ± 0.4b
Aromatics/Cyclics:						
2,4-Di-tert-butylphenol	Phenolic-like, leather-like	1522	0 ± 0b	0 ± 0b	0.7 ± 0.1a	0 ± 0b
Ethylbenzene	Strong	878	23.6 ± 2.4a	21.8 ± 1.3a	16.6 ± 0.2b	15.1 ± 1.0b
Mesitylene	Pesticide	1005	9.4 ± 1.5b	13.0 ± 0.9a	9.7 ± 0.1b	9.4 ± 0.5b
Toluene	Sweet	771	91 ± 2a	99 ± 3a	92 ± 2a	92 ± 1a
p-Xylene	Cold meat fat, metal, sweet	902	12.1 ± 0.4b	16.7 ± 1.3a	12.1 ± 0.3b	11.1 ± 0.4c
Esters:						
Acetic acid, butyl ester	Ethereal, solvent, fruity	817	2.0 ± 0.2a	1.3 ± 0.1b	1.9 ± 0.4a	1.4 ± 0.0b
Acetic acid, hexyl ester	Fruity, green, apple, sweet	1014	2.2 ± 0.2ab	2.7 ± 0.2a	2.1 ± 0.1b	0.6 ± 0.1c
Acetic acid, methyl ester	Solvent-like, fruity	526	7.3 ± 1.1a	2.3 ± 0.4c	4.6 ± 0.5b	5.4 ± 0.7ab
Butanoic acid, methyl ester	Fruity, apple, sweet, banana,	721	0 ± 0c	0 ± 0c	1.7 ± 0.1b	2.6 ± 0.4a
Butyl benzoate	Mild, amber, balsam, fruity	1390	0 ± 0c	1.3 ± 0.1b	1.8 ± 0.0a	1.6 ± 0.2ab
Decanoic acid, methyl ester	Oily, winey, fruity, floral	1326	0.2 ± 0.0c	0.3 ± 0.0bc	0.5 ± 0.0a	0.3 ± 0.0ab
Hexadecanoic acid, methyl ester	Oily, waxy, fatty, orris	1929	0.3 ± 0.1b	0.7 ± 0.1a	0.4 ± 0.1b	0.7 ± 0.0a
Isopropyl myristate	Faint, oily, fatty	1825	0 ± 0b	0.2 ± 0.0a	0 ± 0b	0 ± 0b
Methyl isovalerate	Strong, apple, fruity, pineapple	777	0.8 ± 0.0d	1.6 ± 0.2c	5.7 ± 0.6b	9.2 ± 0.1a
Nonanoic acid, methyl ester	Sweet, fruity, pear, waxy	1226	0.7 ± 0.1b	1.0 ± 0.2a	0.5 ± 0.1c	0.3 ± 0.0d
Furans:						
Furan, 2-methyl-	Ethereal, acetone, chocolate	611	19 ± 1b	86 ± 5a	9 ± 1c	4 ± 0d
Furan, 2-pentyl-	Fruity, green, earthy, beany	995	5.1 ± 0.6a	2.3 ± 0.3b	2.8 ± 0.2b	4.5 ± 0.3a
Ketones:						

(*continued on next page*)

Table 3 (*continued*)

Volatile compound	Odor description	RI exp.	Pea flour	PPI_Ctrl	PPI_SC1	PPI_SC2
2,3-Butanedione	Buttery, sweet, creamy, pungent, caramellic	589	0 ± 0c	0 ± 0c	21 ± 2b	64 ± 1a
2,3-Octanedione	Dill, asparagus, cilantro, herbal	988	1.1 ± 0.1d	2.5 ± 0.1c	8.6 ± 0.2b	14.9 ± 1.2a
2,3-Pentandedione	Pungent, sweet, butter, creamy	697	0 ± 0b	0 ± 0b	69 ± 7a	0 ± 0b
2-Butanone	Acetone, ethereal, fruity, camphoreous	602	2.3 ± 0.1c	3.8 ± 0.1b	4.3 ± 0.2a	3.9 ± 0.1b
2-Heptanone	Cheese, fruity, coconut	896	1 ± 0d	17 ± 1c	78 ± 1a	38 ± 1b
2-Nonanone	Fresh, sweet, green, weedy	1095	0 ± 0d	2 ± 0c	58 ± 0a	44 ± 2b
2-Octanone	Earthy, weedy, natural	995	0 ± 0c	20.6 ± 2.4a	6.3 ± 0.1b	6.6 ± 0.3b
2-Pentadecanone	Fresh, jasmin, celery	1706	0 ± 0b	0 ± 0b	0 ± 0b	0.2 ± 0.0a
2-Pentanone	Sweet, fruity, ethereal, wine	686	0 ± 0c	0 ± 0c	2.9 ± 0.2b	3.7 ± 0.2a
2-Pentanone, 4-methyl-	Sharp, solvent, green, herbal	739	35.9 ± 1.8a	1.7 ± 0.2b	1.6 ± 0.2b	1.3 ± 0.2b
3-Octen-2-one	Earthy, spicy, herbal, sweet	1047	0 ± 0c	0 ± 0c	1.8 ± 0.1b	4.3 ± 0.4a
5,9-Undecadien-2-one, 6,10-dimethyl-, (*E*)-	Green, hay, magnolia	1457	0.8 ± 0.0c	2.9 ± 0.3b	2.9 ± 0.0b	4.5 ± 0.3a
5-Hepten-2-one, 6-methyl-	Citrus, green, musty	990	0 ± 0d	9.8 ± 0.4c	13.4 ± 1.1b	15.4 ± 0.8a
Acetoin	Sweet, buttery, creamy, dairy	713	0 ± 0c	0 ± 0c	59 ± 8b	137 ± 18a
Acetone	Solvent, ethereal, apple, pear	501	13.4 ± 1.7b	20.5 ± 3.8a	24.1 ± 3.4a	24.2 ± 0.8a
Other:						
Trichloromethane	Hay	622	42.2 ± 0.8a	27.2 ± 0.6b	9.8 ± 1.5c	8.6 ± 0.3c
Pyrazines:						
Pyrazine, 2-methoxy-3-(2-methylpropyl)-	Bell pepper, earth, floral, green pepper	1184	0.4 ± 0.1c	3.0 ± 0.1a	2.4 ± 0.1b	2.4 ± 0.1b
Sulfur compounds:						
Carbon disulfide	Vegetable, sulfide	540	0.8 ± 0.1d	1.3 ± 0.0c	3.0 ± 0.3b	4.0 ± 0.1a
Terpenes/Terpenoids:						
Carvone	Minty, licorice	1263	0 ± 0b	0 ± 0b	0 ± 0b	0.3 ± 0.0a
Limonene	Citrus, herbal, terpenic	1039	5.3 ± 0.6a	2.8 ± 0.1b	1.3 ± 0.0d	1.9 ± 0.2c
o-Cymene	Fresh, citrus, terpene	1035	6.2 ± 1.0a	7.3 ± 0.4a	6.3 ± 0.0a	6.8 ± 0.3a
α-Pinene	Fresh, camphor, sweet, pine	942	11.1 ± 1.0d	23.1 ± 1.4a	19.8 ± 0.9b	17.2 ± 0.3c
β-Ocimene	Citrus, tropical, green, terpene	1018	8.2 ± 1.4a	5.5 ± 0.3b	3.8 ± 0.3c	3.8 ± 0.4bc

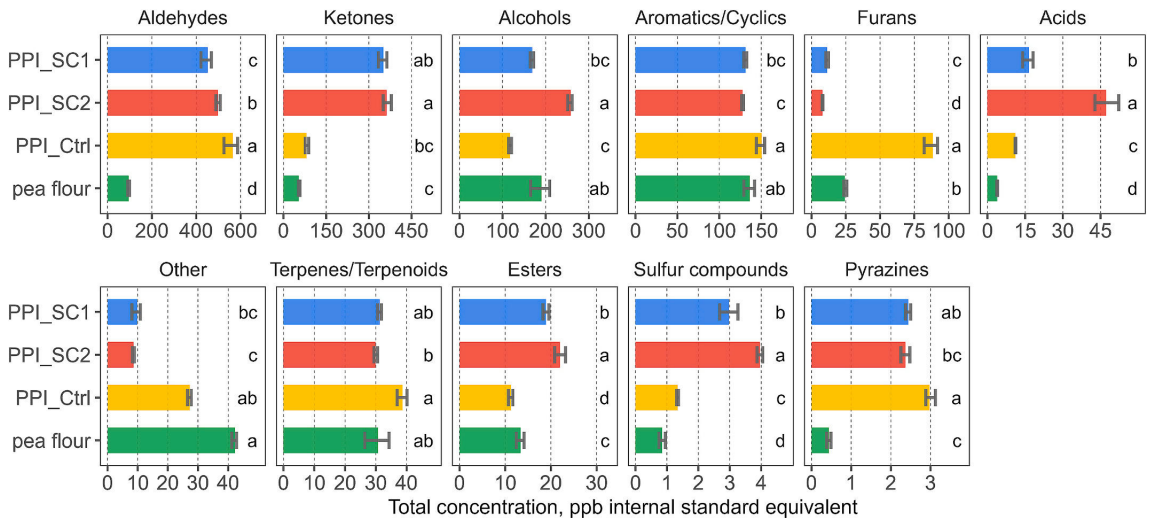

Fig. 3. Total content of volatile compounds in pea protein isolates (PPI) precipitated using a starter culture (SC) or chemically (Ctrl) in comparison to the initial pea flour. The error bars show 95 % confidence intervals (analytical n = 3). Samples not sharing a letter within a panel are statistically significantly different.

panel.

3.5. Sensory characteristics

The sensory properties of PPIs were statistically significantly different in most attributes, except for sour, sweet, and salty taste (Fig. 4). No off-odors or off-flavors were detected, and powder graininess was not perceived. Fermentation significantly reduced pea-like odor and flavor, which was also noted in preliminary experiments, confirming that these results were repeatable. The score for pea-like odor of PPI_Ctrl was 7.4, while PPI_SC1 and PPI_SC2 scored 5.6 and 4.4, respectively. Similar trends were observed in pea flavor and overall flavor intensity. This decrease can be attributed to the lower total concentration of aldehydes, which are the main contributors to "green" off-

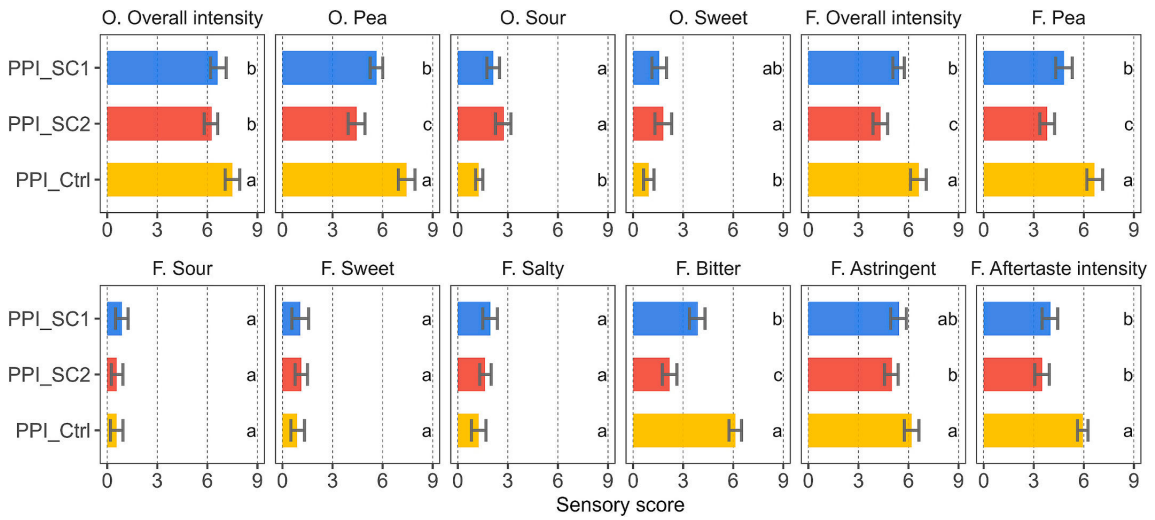

Fig. 4. Average sensory scores for pea protein isolates (PPI) precipitated using a starter culture (SC) or chemically (Ctrl). The error bars show 95 % confidence intervals (analytical $n = 16$). O—odor, F—flavor.

notes together with methoxypyrazines (Liu et al., 2023; Zhogoleva et al., 2023). Interestingly, PPI_SC2 exhibited the lowest pea-like characteristics, despite having a higher content of aldehydes and alcohols than PPI_SC1. This discrepancy may be explained by the slightly lower content of pyrazines and a masking effect of some other volatiles, which has been proposed by Ben-Harb et al. (2019). The reduction of pea-like off-notes through fermentation has been previously reported. For instance, García Arteaga et al. (2021) observed a significant reduction in pea-like aroma and flavor after fermentation with *Lpb. plantarum*, and good results were also achieved with *Lactobacillus perolens*, *Lactobacillus casei*, *Lactobacillus fermentum*, and *P. pentosaceus*.

The fermented PPIs exhibited higher sweet and sour odor intensities than PPI_Ctrl. This could be attributed to acetic and hexanoic acids, as well as "sweet" and "fruity" aldehydes like benzaldehyde, benzeneacetaldehyde, and branched-chain butanals, which were produced during fermentation.

The most notable effect of fermentation was seen in bitterness, which was three times lower in PPI_SC2 than in PPI_Ctrl. Bitterness in peas is primarily caused by saponins, although bitter peptides and free fatty acids also contribute (García Arteaga et al., 2021; Gläser et al., 2021; Heng et al., 2006; Mittermeier-Kleßinger et al., 2021). Thus, fermentation has likely reduced some of these compounds. Effective reduction of PPI bitterness has been previously observed after fermentation with L. *casei* and *Lpb. plantarum* (García Arteaga et al., 2021). Unlike bitterness, fermentation decreased astringency by only approximately one score point. Although LAB may have degraded some astringent molecules, such as phenolic compounds, astringency is also an intrinsic property of many plant proteins themselves due to their high surface hydrophobicity and tendency to aggregate into larger particles and bind to salivary proteins, thereby decreasing lubrication in the mouth (Sarkar, 2024).

Even though SC2 resulted in better sensory properties, fermentation with both SCs significantly enhanced the sensory properties of pea isolates by reducing undesirable flavors, especially bitterness, while slightly increasing sweetness and sourness, without introducing any negative sensory attributes.

3.6. Techno-functional and physicochemical properties

Fermentation altered the techno-functional and physicochemical properties of PPIs, as shown in Table 4. Visually, the fermented PPIs appeared more brownish in color. The lightness (L*) of the fermented samples statistically significantly decreased, whereas redness (a*) increased, with the largest deviation from PPI_Ctrl observed in PPI_SC2. Yellowness (b*) remained unchanged. These findings can be attributed to the elevated temperature during fermentation and the extended process duration. Higher temperature can induce the Maillard reaction, caramelization, and oxidation of phenolic compounds and lipids, resulting in a darker color (Sharan et al., 2021). Because PPI_SC2 was kept at 40 °C longer, its color appeared more brownish than PPI_SC1. A decrease in L* and an increase in a* after fermentation of pea protein have been previously reported (Kaleda et al., 2020; Shi et al., 2021).

The water solubility index (WSI), which quantifies the total powder solubility, decreased by approximately 20 % after fermentation (Table 4). This decline may have resulted from prolonged exposure to higher temperature and the isoelectric pH point, which promotes aggregation of protein particles, thereby decreasing their solubility

Table 4
Color, physicochemical, and techno-functional properties of pea protein isolates (PPI) produced by fermentation with starter cultures (SC) compared to control (Ctrl) precipitated chemically shown as mean values on the dry matter basis with standard deviations (analytical $n = 3$). Values in a row not sharing a letter are statistically significantly different.

Property	PPI_SC1	PPI_SC2	PPI_Ctrl
L*	70.76 ± 0.70b	67.04 ± 0.24c	73.86 ± 0.42a
a*	2.87 ± 0.12b	3.65 ± 0.10a	2.24 ± 0.14c
b*	21.74 ± 0.22a	20.39 ± 0.06a	22.02 ± 0.68a
Water solubility index, %	55.8 ± 0.2b	54.2 ± 0.5b	74.0 ± 1.4a
Water holding capacity, g H_2O g^{-1}	1.53 ± 0.02b	1.68 ± 0.03a	0.78 ± 0.02c
Oil holding capacity, g oil g^{-1}	1.66 ± 0.01ab	1.70 ± 0.02a	1.65 ± 0.02b
Surface hydrophobicity, μg CBBG	23.0 ± 0.6a	21.8 ± 0.3a	21.8 ± 0.9a
Foaming capacity, %	66.7 ± 1.1b	83.3 ± 1.1a	45.5 ± 2.2c
Foaming stability, %	21.1 ± 1.1a	15.4 ± 1.1b	16.9 ± 2.2b
Emulsion activity, %	37.8 ± 1.0a	38.3 ± 0.0a	37.2 ± 1.0a
Emulsion stability, %	88.2 ± 2.7b	92.7 ± 2.5ab	97.0 ± 2.6a
In vitro protein digestibility, %	77.1 ± 1.1b	76.9 ± 0.4b	79.7 ± 0.3a

(Kinsella & Melachouris, 1976). The presence of LAB biomass and their metabolic products (e.g. exopolysaccharides) may have also enhanced interactions between proteins, further decreasing WSI. Nevertheless, our PPIs exhibited higher WSI values than the 10–45 % range reported for commercial PPIs that undergo high-temperature drying (Jakobson et al., 2023). Other studies have also observed that solubility of pea protein decreased after fermentation, attributing this decrease to partial hydrolysis and changes in protein structural configuration that exposed hydrophobic residues and increased aggregation (Kumitch et al., 2020; Shi et al., 2021).

The water holding capacity (WHC) of the fermented samples was twice as high as that of PPI_Ctrl. This is related to the decrease in WSI and the measurement method. Because water is added in excess, only the undissolved part is capable of retaining it. In this way, highly soluble materials exhibit near-zero WHC, resulting in a negative correlation between WHC and WSI (Jakobson et al., 2023). Consequently, the WHC values of our PPIs fell within the lower range of 0.9–3.5 g H_2O g^{-1} reported for commercial PPIs, which also had lower WSI values (Jakobson et al., 2023). In a study by Shi et al. (2021), in which PPI was fermented with *Lpb. plantarum*, the WHC initially decreased until 15 h, then began to increase, but after 30 h still remained 40 % lower than before fermentation.

The oil holding capacity (OHC) of all three PPIs was approximately 1.7 g oil g^{-1}, and the changes due to fermentation were very small. In contrast, Shi et al. (2021) reported that OHC during fermentation had the opposite trend to WHC and initially increased up to three times, but after 10 h began to decrease, and at the end of fermentation was still higher than at the start. Our OHC values were considerably higher than the average OHC of 1.1 g oil g^{-1} found in commercial PPIs (Jakobson et al., 2023). This disparity can be attributed to the effect of lyophilization and the physical entrapment of oil between fine powder particles in comparison to commercial PPIs, which have larger spherical granules produced by spray drying (Kaleda et al., 2020).

The protein surface hydrophobicity, as measured by the amount of CBBG dye bound and precipitated by the protein in suspension, was not affected by fermentation. An increase in OHC is often attributed to changes in protein conformation that expose hydrophobic residues on the protein surface (Kumitch et al., 2020). However, the lack of notable changes in surface hydrophobicity and OHC suggests that both SCs and fermentation conditions used in this study had minimal impact on the protein itself.

Fermentation noticeably improved foaming capacity (FC), particularly in PPI_SC2, corroborating the findings of Shi et al. (2021), who attributed the improvement in FC and foaming stability (FS) to reduced protein solubility, which in turn facilitated a more stable air-bubble interface. In contrast, the FS of our PPIs showed minimal change. Pei et al. (2022) observed that pea flour fermented by L. *rhamnosus* had a significantly lower FC, while FS remained unaffected. The decline in FC was attributed to the exposure of hydrophobic groups, which hindered protein migration to the air-water interface, limiting foam formation. The aggregation between LAB cells, proteins, and fermentation by-products was also hypothesized to impair the foaming properties (Pei et al., 2022). Compared to commercial PPIs, PPI_Ctrl exhibited typical FC, whereas fermented PPIs matched the best foaming proteins such as canola, chickpea, and potato (Jakobson et al., 2023).

The emulsion activity (EA) remained unchanged, but the emulsion stability (ES) of the fermented PPIs was a little lower. This was unsurprising, since emulsifying properties depend on protein surface hydrophobicity, which was unaffected by fermentation. Our EA and ES values were similar to those reported for protein-rich pea flour fermented by *Aspergillus oryzae* and *Aspergillus niger*, which were 38 % and 95 %, respectively (Kumitch et al., 2020). Similarly, Shi et al. (2021) found no significant changes in the emulsifying properties of PPI over 30 h of fermentation with L. *rhamnosus*.

The in vitro protein digestibility (IVPD) after fermentation was statistically significantly reduced, from around 80 % to 77 %. In a previous study by Çabuk et al. (2018), pea protein concentrate was fermented using *Lpb. plantarum*, and IVPD increased from 80 % to 87 % within the first 5 h but decreased to 83 % by 11 h. This effect was linked to the concurrent reduction of trypsin inhibitors, which improves enzymatic activity, and the release of tannins and phenolic compounds from the lignocellulosic matrix, which can bind to proteins, thereby reducing their availability for proteolysis. However, in our study, the insoluble matrix was removed before fermentation, therefore the decrease in IVPD may be rather related to the decrease in WSI, possibly caused by partial protein aggregation. Regardless, the observed small difference of 3 % may not have any meaningful metabolic or physiological effects.

4. Conclusions

We successfully applied fermentation by lactic acid bacteria to acidify and precipitate solubilized pea protein, thereby replacing the chemical acidification step in the conventional pea protein isolation method. We demonstrated that the pea protein solution had a sufficient concentration of sugars to support efficient lactic acid bacteria growth and lactic acid production, which reduced the pH to the isoelectric point of pea protein within 4–7 h. The protein yield and recovery were unaffected, but the properties of the resulting pea protein isolates differed from those of the chemically precipitated control and depended on the chosen starter culture. Fermentation decreased lightness, water solubility index, emulsion stability, and in vitro protein digestibility, while increasing redness, water holding capacity, and foaming capacity. It had little to no effect on oil holding capacity, protein surface hydrophobicity, foaming stability, and emulsion activity. Notably, fermentation significantly improved the sensory properties by reducing undesirable bitterness, pea-like flavors and odors, which could be attributed in part to the decrease in aldehydes possessing “green” odors, including hexanal. Our results could be useful in developing new plant protein isolates with improved sensory characteristics and certain techno-functional properties, thereby expanding their applicability in sustainable meat and dairy alternatives.

CRediT authorship contribution statement

Aleksei Kaleda: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Navratan Sharma:** Methodology, Investigation, Formal analysis, Data curation. **Kadi Jakobson:** Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition. **Irina Stulova:** Writing – original draft, Investigation. **Sirli Rosenvald:** Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Ethical statement

The internal scientific research committee of TFTAK AS (Tallinn, Estonia) approved the design of the sensory study. Participants were selected from an internal pool of highly trained evaluators who volunteered for sensory assessments. The selected panelists had prior experience evaluating plant protein powders and provided written consent. Before the study began, participants were informed about its purpose and procedures. Participation was voluntary, and panelists were free to withdraw from the test at any time. All panelists were in good health and had no known allergies to the studied material. Participants were assured of the confidentiality of their data.

Funding details

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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.

Acknowledgments

The authors express gratitude to colleagues from TFTAK, who assisted in the experimental work and conducted the analyses. Marie Kriisa—starter culture selection, Karel Talvistu—protein extraction, Viktoria Soboleva—chemical and techno-functional analyzes, Aaro Videvik—volatiles, Marina Junusova—sugars and organic acids, Helen Vaikma and Maarja Maasikmets—sensory assessment. We also thank two anonymous reviewers who critically evaluated this paper.

Data availability

The data are contained within the article; additional information is available upon request.

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Appendix 4

Publication IV

Latrofa, V., Kaleda, A., De Angelis, D., **Jakobson, K**., Squeo, G., Caponio, F., Pasqualone, A., & Summo, C. (2026). Enhancing texturization of upcycled durum wheat meal protein through pH adjustment and low-moisture extrusion optimization. Applied Food Research, 6(1), 101925. doi.org/10.1016/j.afres.2026.101925

Applied Food Research 6 (2026) 101925

Contents lists available at ScienceDirect

Applied Food Research

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

Enhancing texturization of upcycled durum wheat meal protein through pH adjustment and low-moisture extrusion optimization

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ARTICLE INFO

Keywords:
Durum wheat meal
By-product upcycling
Low-moisture extrusion
Texturized vegetable protein
Meat analog
Physicochemical properties

ABSTRACT

Dry-fractionated, defatted durum wheat meal protein (DWMP) is an emerging upcycled food ingredient produced from by-products generated during semolina production. In our previous work, DWMP demonstrated potential for use in texturized vegetable protein (TVP) formulations. However, the resulting texture was suboptimal, suggesting the need for optimization of extrusion parameters. Therefore, the effects of three extrusion parameters (pH 6.9 and 7.5, screw speed 400 rpm and 600 rpm, and moisture content 28% and 32%) were evaluated during the production of TVP formulated with a pea protein isolate (PPI) and DWMP (75:25 w/w). Specifically, pH shifting was chosen as an extrusion variable having a significant impact on texture formation. Textural, physicochemical, and sensory properties of the extrudates were assessed using response surface methodology with a 2^3 reduced factorial design. Higher screw speed increased the water-holding capacity (WHC) (on average, from 2.7 to 3.4 g H_2O/g) and specific volume (2.2→2.6 mL/g), while decreasing the water-solubility index (WSI) (14.1→11.9%) and hardness (4522→2763 g). Higher moisture content led to increased hardness (3255→4128 g) and decreased springiness (49.8→44.0%), and sensory perception of fibrousness (1.4→1.6 score). At higher pH, the WHC improved (2.5→3.4 g H_2O/g), thereby leading to higher sensory moistness (5.4→6.3 score), and lower sensory hardness (2.8→2.1 score) of the extrudates. Overall, the findings demonstrate that extrusion conditions can be modulated to improve the physicochemical and sensory characteristics of the products, supporting the valorization of durum wheat milling side-streams in sustainable plant-based meat applications.

1. Introduction

The milling process of durum wheat (*Triticum turgidum* L. var. durum) produces substantial quantities of by-products consisting of germ and bran fractions, which represent an underutilized source of protein with strong potential for circular food system applications. Our recent work (Latrofa et al., 2025) investigated the effect of dry-fractionated durum wheat meal protein (DWMP) incorporation on volatile compounds, and textural and sensory properties of pea-based texturized vegetable proteins (TVP), also known as meat analogs. Compared to conventional wheat-derived proteins, DWMP is obtained through milling and air classification of defatted germ, bran, and debranning fractions (Squeo et al., 2023). DWMP increased expansion, modified texture attributes such as springiness and cohesiveness, and reduced key volatile compounds associated with green or beany notes, showing that DWMP can serve as a secondary protein source for meat analog formulations. While these results confirmed the potential of DWMP for texturized applications, the resulting structures still displayed some issues, suggesting that the full texturization capacity of DWMP may depend on specific processing conditions and requires further investigation (Latrofa et al., 2025). Based on this premise, understanding how extrusion variables shape the texturization behavior of the raw materials becomes essential.

In the current research, we focus on low-moisture extrusion (LME),

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https://doi.org/10.1016/j.afres.2026.101925
Received 19 December 2025; Received in revised form 2 March 2026; Accepted 21 March 2026
Available online 22 March 2026
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since it is one of the most widely applied technologies for producing TVP (De Angelis et al., 2024c). LME allows plant protein blends to be transformed into porous, fibrous structures that can be easily rehydrated and subsequently incorporated into final products, making it accessible to a wide range of producers through its modest equipment demands and adaptability to different raw materials (Mandliya et al., 2022; do Carmo et al., 2023). In this process, a protein-rich raw material is mixed with water (below 40%), melted, and under high pressure pushed through a small opening using a set of screws (De Angelis et al., 2024c; Kaleda et al., 2021). The typical fibrous structure obtained in LME is due to the alignment of denatured protein chains under a strong shear flow at high temperature (Van der Sman & van der Goot, 2023). The texturing ability of proteins during LME is governed not only by their intrinsic molecular characteristics but also by the key process variables, including moisture content, screw speed, and temperature, overall affecting the final textural quality of TVP (Beck, Knoerzer & Arcot, 2017; Vatansever, Tulbek & Riaz, 2020). Moisture content is relevant because water acts as a plasticizer, influencing viscosity of the melt (De Angelis et al., 2023; Van der Sman & van der Goot, 2023). The mechanical action of the screws contributes to protein alignment during shear flow in the screw section (Van der Sman & van der Goot, 2023). Furthermore, the pH of the raw materials and the presence of ions can influence intermolecular interactions across macromolecules and thereby affect fiber formation (Cheftel, Kitagawa & Quéguiner, 1992). Additives that change ionic strength and pH, such as NaCl, $KHCO_3$, and $CaCO_3$, are used industrially (Patent US 2015/0296,835; Van der Sman & van der Goot, 2023). Nisov et al. (2022) visually observed fibrous structure formation while varying the pH in high-moisture extrusion, finding that the shift from pH 5 to pH 7 improved texturization due to higher reactivity of disulfide bonds at higher pH. Muhialdin and Ubbink (2023) varied the pH from 5 to 9 in high-moisture extrusion of a pre-hydrated pea protein isolate, reporting a significant decrease in hardness of the extrudates while the water holding capacity (WHC) increased. However, while the role of pH has been investigated in high-moisture extrusion conditions (Muhialdin & Ubbink, 2023; Nisov et al., 2022), its impact under low-moisture conditions has not yet been systematically explored.

Considering the central role of extrusion conditions in shaping the physicochemical, textural, and sensory properties of texturized vegetable proteins, the present study aimed to evaluate the effects of screw speed, feed moisture, and pH on TVP produced from a blend of DWMP and pea protein isolate.

2. Materials and methods

2.1. Materials

The DWMP was provided by Casillo Next Gen Food srl (Corato, Italy). The durum wheat by-product consisted of a mixture of germ, bran, and debranning fractions, which was defatted as described in Squeo et al. (2022) and then processed through a dry fractionation technique as explained in Squeo et al. (2023). The obtained DWMP fraction contained 28.53 g/100 g protein, 8.53 g/100 g fibers, 6.24 g/100 g ash, 0.81 g/100 g lipid, and 55.89 g/100 g carbohydrates (dry matter basis). The chemical composition was determined as reported in Section 2.2. Commercial yellow pea protein isolate (PPI) was purchased from Caremoli S.p.A (Monza, Italy) and had 80.0 g/100 g protein, 10.0 g/100 g fat, 2.0 g/100 g carbohydrates, and 8.0 g/100 g fibers (dry matter basis). Dry-fractionated commercial brown pea starch concentrate (PSC) was purchased from Aloja-Starkelsen (Aloja, Latvia) and had 11.5 g/100 g protein, 1.4 g/100 g fat, 77.1 g/100 g carbohydrates, and 10.0 g/100 g fibers (dry matter basis). All the chemical compositions were given by the producers. All the reagents were purchased from Merk KGaA (Darmstadt, Germany). KOH (E525) was food grade.

2.2. Chemical analysis, preparation of the blends, and experimental design

The protein content ($N \times 6.25$) of the raw materials was determined using the Kjeldahl method as described in AOAC 979.09 (AOAC 2006). The lipid content was determined using a Randall apparatus (SER 148 extraction system, Velp Scientifica s.r.l., Usmate Velate, Italy) following the method AOAC 945.38F with diethyl ether as an extraction solvent (AOAC 2006). Ash determination was performed as described in the method AOAC 923.03 (AOAC 2006). Carbohydrates were determined by difference from protein, lipid, fiber, and ash contents. All determinations were performed in triplicate.

A blend of pea protein isolate and DWMP at a ratio of 75:25 (PPI_DWMP), corresponding to a protein content of 65.7%, was used for the extrusion trials. This formulation was selected based on our previous work (Latrofa et al., 2025), which indicated that DWMP levels between 20% and 30% are a good compromise in terms of structural and physicochemical properties. A 2^3 reduced factorial experimental design was used to plan extrusion experiments, as shown in Table 1. The order of experiments was randomized, and a replicate of one test (i.e., S7_1 and S7_2) was made to increase the degrees of freedom for estimation of variance and statistical significance. The independent variables were total moisture content (28% and 32%), screw speed (400 rpm and 600 rpm), and pH (6.9 and 7.5). The ranges were determined in preliminary trials. The pH of all the extrudates was measured after the extrusion, as reported in Section 2.4. The native samples had a pH of 6.9 ± 0.02, and for this reason, it was chosen as the lower limit in the experimental design. Then, pH was adjusted to 7.5 ± 0.04 by adding 1.5% of a KOH solution (10% w/w) into the extruder. This upper limit was chosen because higher pH values negatively affected the overall product quality in terms of texture and color during preliminary trials. KOH was used due to its high solubility and common use in the food industry to modify the pH. For comparison, two reference samples were prepared from acknowledged recipes: a pure pea protein isolate (PPI), and a mixture of pea protein isolate with pea starch concentrate (80:20) (PPI_PSC) containing 66.3% proteins. These recipes were extruded at native pH, following the extrusion conditions already standardized from the laboratory of the TFTAK (Tallinn, Estonia) that were: 36% of moisture and 320 rpm for PPI; 26% of moisture and 700 rpm for PPI_PSC. These reference samples imitated typical TVP available on the market. The extrusion conditions for references were chosen to obtain desirable texture, similar to commercial TVPs. However, since the protein type was different from the experimental samples, their optimal extrusion conditions differed from the conditions applied to the experimental samples.

2.3. Extrusion process

The extrusion process was conducted using a co-rotating intermeshing twin-screw extruder, KETSE 20/40 (Brabender GmbH,

Table 1
The experimental design for extrusion of texturized vegetable proteins. The order of the experiment was randomized during trials.

Trial	Total moisture (%)	Screw speed (rpm)	pH
S1	28	400	6.9
S2	28	600	6.9
S3	32	400	6.9
S4	32	600	6.9
S5	28	400	7.5
S6	28	600	7.5
S7_1	32	400	7.5
S7_2	32	400	7.5
S8	32	600	7.5

Samples were produced with pea protein isolate and durum wheat meal protein (75:25 w:w).

Duisburg, Germany), with a temperature profile of 40/76/122/135/152/154 °C that was chosen after preliminary experiments. Each powder was previously calibrated to determine the powder mass flow rate in the volumetric feeder. Water and the KOH solution were added through separate ports with two calibrated peristaltic pumps. The specific mechanical energy (SME) was calculated as described by Kaleda et al. (2021). The actual total mass flow rate inside the extruder (4.4–4.8 kg/h) was calculated by measuring the weight of extrudates produced in three minutes and the weight of liquids (water, KOH) pumped into the extruder during the same period, taking into account the moisture contents of the entering powder and the exiting extrudate. The diameter of the die hole was 1.6 mm. The extrudates were collected after stabilization of the process, when the temperature, die pressure, and screw torque recorded every second by the extruder were constant. The samples were dried in an oven for 50 min at 70 °C until the water activity dropped below 0.6, to prevent microbial growth, and packed into zip-lock bags (Latrofa et al., 2025).

2.4. pH, physicochemical and textural properties

The pH of the extrudates was determined by homogenizing 5 g of extrudates and 25 g of distilled water using a Polytron PT 2100 (Kinematica AG, Malters, Switzerland).

Water holding capacity (WHC), water solubility index (WSI), and oil holding capacity (OHC) were measured as described by Kaleda et al. (2021). WHC and WSI were measured for raw materials before extrusion, and for both milled and whole TVP after extrusion. OHC was measured for raw materials before extrusion and for milled extrudates. To obtain a milled extrudate, it was milled with a coffee mill and then sieved with a 0.6 mm sieve to obtain a uniform particle size during the analysis.

The specific volume was assessed as described in Latrofa et al. (2025).

Texture profile analysis (TPA) of the TVP was carried out using a TA. XT2i Texture Analyzer (Stable Micro Systems, Godalming, UK) equipped with a 75 mm compression plate and a 50 kg load cell. The analysis was conducted as described in Kaleda et al. (2021) with some modifications. Whole extrudates were rehydrated in tap water at 60 °C for 60 min and then blotted dry with paper towels before the analysis. 1.6 g of extruded pieces were compressed twice with the following conditions: 3 mm/s pre-test, 1 mm/s test, 10 mm/s post-test speed, 70% compression, and 2 s between the two compressions. To standardize the amount of extrudates in the analysis, a quantity of 1.6 g was chosen to have a uniform layer on the plate. Hardness (g), resilience (%), cohesion, springiness (%), and chewiness were calculated using the texture analyzer software (Exponent Connect version 8.1.9.0, Stable Micro Systems, Godalming, UK).

2.5. In-vitro protein digestibility (IVPD)

The *in-vitro* protein digestibility (IVPD) of the powders and milled extrudates was determined adapting the method described in Espinosa-Ramírez et al. (2018). Briefly, 1 mL of a protein dispersion containing 6.25 mg of protein/mL was heated at 37 °C in a water bath. The pH was adjusted to 8.00 with 0.1 N NaOH and/or 0.1 N HCl during stirring. An enzyme solution with trypsin at a concentration of 1.6 mg/mL was maintained at +4 °C until use, and the pH was adjusted to 8.00 with 0.1 N NaOH and/or 0.1 N HCl. The trypsin activity was 13,766 BAEE units/mg protein. 0.1 mL of the enzyme solution was added to the protein suspension preheated in a water bath, and the pH was recorded after 10.0 min after the addition of the trypsin solution. The IVPD percentage was calculated using the equation $y = 210.464 - 18.1\ x$ from Espinosa-Ramírez et al. (2018), where x is the pH value after 10 min.

2.6. Sensory analysis

Quantitative descriptive sensory analysis was conducted at TFTAK (Tallinn, Estonia) by an internal sensory panel composed of nine panelists. The assessments were held individually in isolated testing booths in a dedicated sensory room following the ISO standard 8589:2007 (ISO, 2007). All panelists, with an average age of 33 ± 9, had previous training and experience in evaluating plant-based extrudates (Latrofa et al., 2025) as described in ISO standard 8586:2012 (ISO, 2012). More details about training and procedure have been previously described (Latrofa et al., 2025). Participants gave written consent to take part in the sensory analysis. The samples were rehydrated in tap water at 60 °C for 60 min and the excess water was blotted dry before the analysis. All samples were coded with random three-digit codes, and the order of assessment was randomized for each assessor according to Williams' Latin Square design to reduce the presentation effect. The samples were served at 21 °C in clear 40 mL plastic cups and presented in sequentially monadic order. The analysis used a 10-point structured scale, where 0—"none", 1—"very weak", 5—"moderate", and 9—"very strong". The scale anchors used for this evaluation are reported in Latrofa et al. (2025). The sensory analysis was conducted according to an internal sensory protocol, which focused mainly on three modalities: odor, taste, and texture. For odor and taste, overall intensity and raw material intensity were evaluated. In texture, fibrousness, springiness, hardness, chewiness, adhesiveness, moistness, and granularity were evaluated. All texture attributes were assessed orally, except for fibrousness, which was evaluated using hands. Data collection was carried out using RedJade sensory software version 6.1 (RedJade Sensory Solutions LLC, Martinez, CA, USA).

2.7. Statistical analysis

The data are presented as means with standard deviations. Statistical significance was evaluated using one-way ANOVA followed by Tukey's HSD test at $\alpha = 0.05$ with Minitab 19 Statistical Software (Minitab Inc., State College, PA, USA). The data was assumed to be normally distributed and have equal variance for the purpose of ANOVA testing. The differences between sample TVP and reference TVP were evaluated using Dunnett's multiple comparisons test at $\alpha = 0.05$. WHC, WSI, OHC, specific volume, and IVPD were measured in triplicate, TPA in eight replicates, and sensory analysis was done in two replicate sessions.

The response surface models for the design of experiments data were calculated using Chemometric Agile Tool version 05.09.2023 (Leardi, Melzi & Polotti, 2013). The fitted model was $Y = \beta_0 + \beta_1 \times_1 + \beta_2 \times_2 + \beta_3 \times_3 + \beta_{12} \times_1 \times_2 + \beta_{13} \times_1 \times_3 + \beta_{23} \times_2 \times_3$, where Y is the response variable, β are model coefficients, and x are encoded [−1, +1] extrusion parameters. The experimental variance and coefficient significance ($p < 0.05$) were estimated by residuals. Model visualization was performed in R version 4.3.0 (The R Foundation for Statistical Computing, Vienna, Austria).

3. Results and discussion

3.1. Physicochemical properties and protein digestibility of raw materials

The physicochemical properties of the raw materials are reported in Table 2. WHC/OHC reflect the ability of hydrophilic/hydrophobic molecules, in particular polysaccharides and proteins, to bind water/oil (De Angelis et al., 2024a; Zhao et al., 2020). The WHC of all three raw materials did not differ, but the OHC was the highest in PPI_PSC, which could be due to its higher starch content. Similar values were previously reported for pea protein ingredients (De Angelis et al., 2024b; Jakobson et al., 2023; Zhao et al., 2020). The WSI measures total powder solubility at room temperature at native pH (Jakobson et al., 2023). The presence of DWMP caused a significant increase in WSI due to the presence of soluble dietary fibers and minerals. Similar conclusion was also shown

Table 2
Physicochemical and digestibility characteristics of the initial raw materials.

Sample	WHC (g H_2O/ g powder)	OHC (g oil/ g powder)	WSI (%)	IVPD (%)
PPI	3.21 ± 0.11[a]	0.98 ± 0.06[b]	7.05 ± 0.10[b]	72.12 ± 0.58[a]
PPI_PSC	3.10 ± 0.06[a]	1.25 ± 0.12[a]	6.51 ± 0.41[b]	72.72 ± 0.18[a]
PPI_DWMP	3.10 ± 0.09[a]	0.99 ± 0.10[b]	13.34 ± 0.43[a]	72.66 ± 0.38[a]

Data are expressed as means ($n = 3$) ± SD. Different letters in the same column indicate significant differences according to the Tukey's HSD test ($p \leq 0.05$). PPI—pea protein isolate; PPI_PSC—mix with pea protein isolate and pea starch concentrate (80:20 w:w); PPI_DWMP—mix with pea protein isolate and durum wheat meal protein (75:25 w:w); WHC—water holding capacity; WSI—water solubility index; OHC—oil holding capacity, IVPD—*in-vitro* protein digestibility.

by Escobedo et al. (2020) in bean flours.

The IVPD of the powders (Table 2) was not statistically different. The reason may lie in the greatly larger proportion of PPI (compared to other powders) in the recipe, which dominated the IVPD properties of all recipes. Protein digestibility is usually associated with amino acid availability (Espinosa-Ramírez et al., 2018), and varies due to the chemical composition, genetic and environmental features, and anti-nutritional factors of the material. Similar values of protein digestibility were found by Teka et al. (2020) in cowpea flours.

3.2. Effect of the processing conditions on the properties of extrudates

Response surface methodology was employed to investigate the effect of independent extrusion parameters on the properties of the extrudates. The fitted models are summarized in Table 3, where the significant coefficients ($p < 0.05$) are shown with an asterisk. Overall, it became clear that the postulated model accurately describes the behavior of most of the responses studied, as evidenced by the explained variance. In a few cases, however, the model did not fit the response, as demonstrated by the negative value of the explained variance. The coefficients of the screw speed and moisture were often found to be significant, suggesting that the setting of these parameters has a remarkable effect on the extrudate characteristics.

In the subsequent sections, the response surfaces are presented. For the physicochemical properties and texture profile analysis, only the models with $R^2 \geq 0.8$ are exhibited (Lundstedt et al., 1998), while for the sensory analysis, the criterion was $R^2 \geq 0.7$, as the data presented high variability. All the models with low explained variance were not taken into consideration. The remaining data is shown in Supplementary materials.

3.2.1. Physicochemical properties and protein digestibility of extrudates

The response surface models of the physical properties of the extrudates are depicted in Fig. 1. WHC was measured on the milled ones (Fig. 1a,b) and on the whole extrudates (Fig. 1c,d). The measurement on the milled extrudate emphasizes the physicochemical characteristics of the sample, while the determination on the whole extrudates also considers capillary entrapment of water within the porous TVP structure. Since TVP are usually rehydrated before their utilization in the final formulation (Lee et al., 2022; Sha & Xiong, 2020), evaluating WHC in whole extrudates provides a more realistic indication of their hydration behavior in final applications. WHC determines the acceptability of extrudates in terms of juiciness (succulence), tenderness, texture, and mouthfeel (Brishti et al., 2021; Bhuiyan et al., 2025; Lee et al., 2022).

As shown by the response surfaces, the WHC of the whole extrudates was differently affected by the processing conditions compared to the milled ones. In whole extrudates, WHC increased significantly ($p < 0.05$) with screw speed at both pH values, with a steeper response at pH 7.5 (Fig. 1d). This is consistent with the well-documented increase in expansion at higher screw speeds (Singh et al., 2024), as larger and more open pores facilitate greater water penetration and entrapment. This interpretation aligns with the increase in specific volume observed and discussed in Section 3.2.2.

For milled extrudates, WHC was influenced by molecular-level changes rather than structural porosity. WHC was affected by all three factors and by their interactions, with pH having the greatest effect among the linear variables. Moreover, from the surface responses in Fig. 1, a change in the slope direction could be seen between pH 6.9 (Fig. 1a) and 7.5 (Fig. 1b), highlighting the interaction between the pH and the moisture content. Increasing moisture acts as a plasticizer, lowering protein denaturation and glass transition temperatures and

Table 3
The coefficients of the response surface models and their statistical significance (* indicates $p < 0.05$).

Analysis	Screw speed	Moisture	pH	Screw speed × Moisture	Screw speed × pH	Moisture × pH	Explained variance, %
WHC whole (g H_2O/g extrudate)	0.41*	−0.06	0.48*	−0.36*	0.13	0.07	96.99
WHC milled (g H_2O/g extrudate)	−0.08*	−0.02*	0.19*	−0.09*	−0.13*	−0.11*	99.86
WSI whole (%)	−1.01*	0.43	0.16	0.17	−0.81	−0.17	81.96
WSI milled (%)	−0.71	−0.26	−0.66	0.00	−0.47	0.13	−52.79
OHC (g oil/g extrudate)	0.05	−0.01	−0.04	−0.01	0.02	0.03	−22.70
IVDP (%)	0.75	0.05	0.18	−0.14	0.30	−0.07	46.67
Hardness (g)	−833*	341*	44	−5	−143	−67	96.02
Resilience (%)	0.41	−1.04	0.52	−0.52	−0.27	1.14	57.72
Cohesion (-)	0.02	−0.02	0.00	−0.007	−0.003	0.02	73.49
Springiness (%)	1.54	−3.09*	−0.19	−0.68	−0.48	2.58	84.21
Chewiness (-)	−145.1	−3.4	7.6	−5.6	−47.6	66.1	73.49
Specific volume (mL/g)	0.21*	−0.06	0.01	−0.09*	−0.05	0.05	95.88
SME (Wh/kg)	17.8*	−16.8*	−3.8	−1.0	−1.0	1.5	97.84
X.Fibrousness (-)	0.08	0.15*	−0.03	0.05	0.03	−3.4	70.97
X.Hardness (-)	−0.04	−0.04	−0.39*	0.11	−0.04	0.07	76.64
X.Moistness (-)	0.35	0.02	0.47*	−0.12	0.03	0.05	72.47
X.Chewiness (-)	−0.04	−0.14	−0.14	0.11	−0.04	0.06	75.80
X.Cohesiveness (-)	−0.07	−0.08	0.02	0.10	0.05	0.10	−87.91
X.Granularity (-)	−0.11	0.01	−0.11	0.04	−0.04	0.04	−19.22
X.Springiness (-)	0.09	−0.04	−0.16	0.06	−0.02	0.07	24.73

SME—specific mechanical energy; WHC—water holding capacity; WSI—water solubility index; OHC—oil holding capacity, IVPD—in vitro protein digestibility. X. indicates the sensory evaluation of textural attributes.

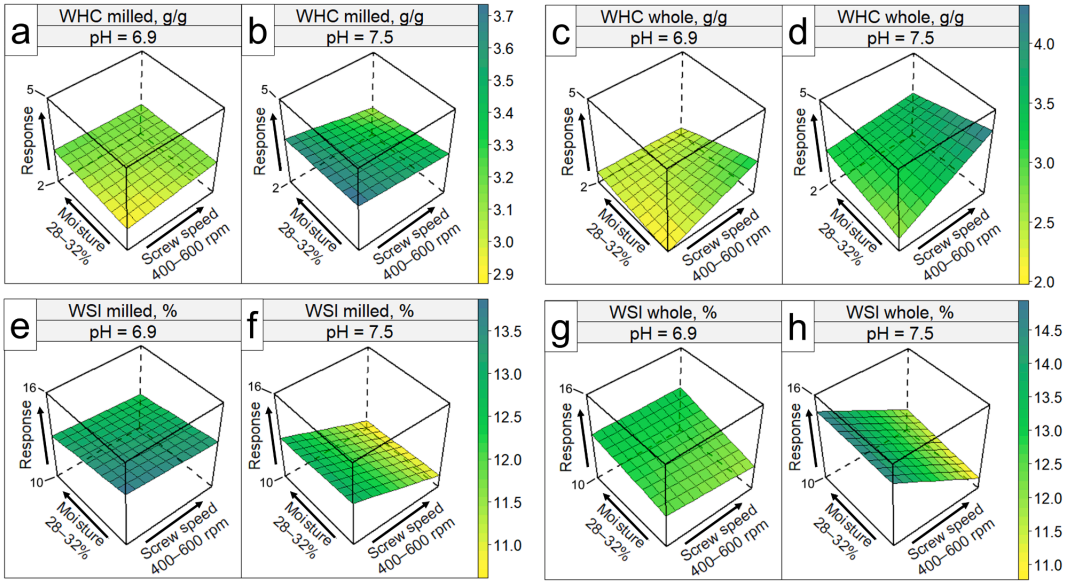

Fig. 1. Response surface plots depicting the variations of WHC milled (a,b) and whole (c,d) and WSI milled (e,f) and whole (g, h) properties of the extrudates produced with different extrusion parameters. WHC—water holding capacity; WSI—water solubility index.

promoting intermolecular bonding (Van der Sman & van der Goot, 2023). A tighter protein network likely reduces the number of accessible hydrophilic sites, explaining the significant decrease in WHC with higher moisture content ($p = 0.03$; Table 3). At pH 7.5, both milled and whole extrudates showed higher WHC. Similar pH-dependent increases have been reported by Muhialdin and Ubbink (2023). In fact, at high pH values, the charge of protein chains is negative, thus hydrophobic interchain interactions are not favored. This loosens the matrix and lowers its mechanical properties, which in turn enhances WHC (Muhialdin & Ubbink, 2023).

In addition, increasing screw speed reduced WHC in milled samples. This behavior was likely due to the more intense shear denatured proteins more strongly, decreasing the availability of hydroxyl and other hydrophilic groups capable of binding water (Kaleda et al., 2021; Zhang et al., 2024). In fact, screw speed and temperature are the main parameters responsible for changing protein molecular conformation (Schmid et al., 2022), supporting this interpretation.

Water absorption is usually inversely correlated with WSI (Fallahi et al., 2013; Jakobson et al., 2023), and an increase in WSI might be related to macromolecular degradation of extrudates and dextrinization of starch (Kaleda et al., 2021). However, the WSI model for the whole extrudates (Fig. 1g,h) suggested that higher screw speed lowered the WSI significantly ($p < 0.05$). The higher expansion at higher screw speed (Section 3.2.2) explains this finding because more water with dissolved material can be entrapped in the pores of the expanded structure. By contrast, the response surface models of milled extrudates (Fig. 1e,f) indicated that the WSI was not affected by the process conditions ($p > 0.05$). Previous studies have reported that pH adjustment using KOH introduces K^+ ions into the system, potentially enhancing protein solubility (Abd Rahim et al., 2024; Khalesi et al., 2024). However, in the present study, no increase in solubility was observed at higher pH values, suggesting that the combined effect of pH modification and the associated increase in ionic strength was not pronounced enough to markedly influence the solubility of the extrudates. In addition, the blends used in extrusion contained two different sources of protein. Wheat proteins have relatively high isoelectric points (pH 7–8) (De Angelis et al., 2024b). The applied pH shift likely brought wheat proteins closer to their isoelectric point, decreasing their solubility, while pea proteins with an isoelectric point around pH 4.8 were shifted further from it and therefore became more soluble, possibly compensating for the decrease in solubility of wheat proteins.

The model for OHC did not fit the data, thus, the explained variance was negative (Table 3) suggesting that this property was unaffected by the extrusion conditions. Compared to the raw material, OHC was significantly enhanced ($p < 0.05$) after the extrusion process (Table 2 and Supplementary material, Table S1). Likewise, Kaleda et al. (2021) found an increase of OHC after extrusion due to protein denaturation that increased the availability of hydrophobic groups. Considering the application in meat alternatives, OHC should be evaluated because usually oils, e.g. sunflower and rapeseed oil, are added to improve the sensory profile and overall acceptability of the final product (Starowicz, Poznar & Zieliński, 2022; Sha & Xiong, 2020; Zhang et al., 2023). Moreover, the meat-like juiciness of meat analogs is determined by their moisture and oil retention both (Zhang et al., 2023).

Similarly, the IVPD of the extrudates was evaluated, but as shown in Table 3 the explained variance was low; thus, the variation of the process parameters did not affect the IVPD. The IVPD of the extrudates ranged from 79.66% to 82.14% (Supplementary material, Table S2) and was significantly higher ($p < 0.05$) than the IVPD of the raw material that was in the range of 72.12–72.66% (Table 2). Phytic acid, trypsin inhibitors, and tannins contained in legumes could decrease protein digestibility. Some heat-labile antinutrients could be reduced during extrusion due to the thermomechanical process (De Angelis et al., 2020, 2024c), hence improving the digestibility compared to the raw material.

3.2.2. *Texture, specific volume, and specific mechanical energy*

The instrumental texture parameters that were significantly affected by the extrusion conditions (screw speed and moisture) were hardness, springiness, and specific volume (Table 3), which are visualized as contour plots in Fig. 2. The data for each sample is presented in Supplementary material, Table S2. Hardness is the maximum force required to compress the sample, whereas springiness indicates how much of the

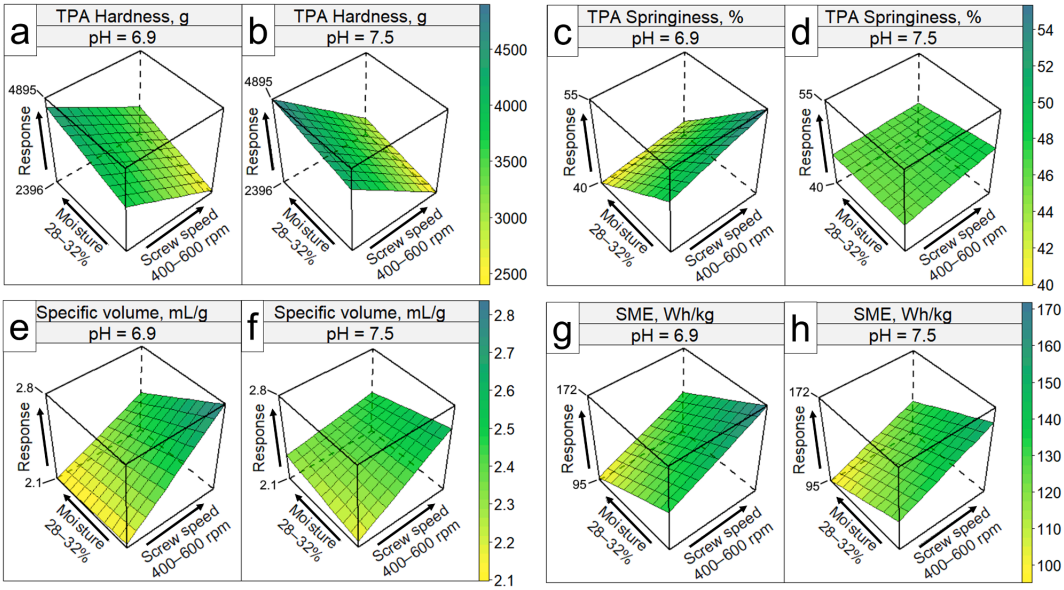

Fig. 2. Response surface plots depicting the variations of texture properties of the extrudates produced with different extrusion parameters. Texture profile analysis (TPA) of hardness (a,b) and springiness (c,d), specific volume (e, f), and specific mechanical energy (SME) (g, h).

sample recovers after the first deformation. The specific volume is indicative of the expansion and density of extrudates (De Angelis et al., 2020).

Higher screw speed lowered hardness, while an increase in moisture content increased hardness instead. A reduction in hardness at higher screw speeds has similarly been reported for low-moisture extrudates (Li et al., 2005). High screw speed enhances the formation of elongated, fibrous structures (Schmid et al., 2022; Wang et al., 2023). Additionally, the greater expansion (i.e., higher specific volume) observed at higher screw speeds (Fig. 2e,f) likely contributed further to the reduction in hardness. In fact, larger pores in the extrudates may have taken up more water during sample preparation, effectively reducing the solids content during TPA measurements. The positive effect of moisture content on protein network formation was already discussed above for WHC. Moreover, the model for springiness identified an interaction between moisture and pH ($p = 0.05$). Specifically, at pH 6.9 springiness decreased with moisture (Fig. 2c), but at pH 7.5 it had no effect (Fig. 2d). As shown in Fig. 2 and Table 3, the specific volume increased by increasing the screw speed, a trend widely reported in extrusion studies (Bisharat et al., 2013; Singh et al., 2024), indicating the expansion of the product.

The specific mechanical energy (SME) expresses the amount of mechanical work input that occurs due to screw rotation. SME is positively correlated with melt viscosity and screw speed, while it is negatively correlated with moisture content (Chen et al., 2023). Indeed, higher moisture content led to lower SME, and higher screw speed increased SME (Fig. 2g, h). Since higher SME is associated with greater expansion (do Carmo et al., 2023), the significant interaction between screw speed and moisture (Table 3) reflects the balance between mechanical energy input and melt plasticization. Although moisture was not significant as a main factor, its plasticizing action may have constrained expansion by facilitating denser bubble formation and stronger intermolecular bonding.

These mechanical measurements of extrudate texture indicated that the applied extrusion conditions can influence both texture and porosity. However, to gain a deeper understanding of the structural differences in the extrudates, future studies should directly assess porosity and texture at the microstructural level using techniques such as scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM).

3.2.3. *Sensory properties of extrudates*

Figure 3 depicts the contour plots of the sensory parameters significantly affected by the process conditions, as evidenced by the coefficients reported in Table 3. The sensory panel assessed the extrudates after rehydration, which is necessary to obtain an elastic meat-like texture (Sha and Xiong, 2020; Lee et al., 2022; Brishti et al., 2021). Fibrousness was significantly higher at higher moisture content ($p < 0.05$), reflecting previous observations concerning the role of water in promoting the protein network. In fact, moisture content promotes disulfide cross-links, hydrogen, ionic, and hydrophobic bond formation (Maung et al., 2020), resulting in a denser structure. A more pronounced anisotropic structure was found at higher moisture content in high-moisture extrudates (De Angelis et al., 2023). Nisov et al. (2022) reported that an increase in pH enhances the reactivity of disulfide bonds at higher pH conditions, promoting fibrous structure formation. However, in our study, fibrousness was not significantly affected by the pH shift.

Interestingly, sensory analysis revealed differences in hardness driven by pH ($p = 0.03$), which were not detected instrumentally. The discrepancy observed between the sensory results and TPA measurements may be explained by several factors. These include lubrication effects occurring during mastication, the temperature in the mouth, the changes in the food microstructure that occur during mastication, and more generally, the dynamic nature of oral processing, which differs substantially from the compression applied in instrumental tests (Nishinari et al., 2019; Nishinari & Fang, 2018; Swackhamer & Bornhorst, 2019). Both analytical methods used for evaluating the texture of TVP are valid, but combining instrumental measurements with sensory evaluation leads to a more comprehensive understanding of the textural properties of TVP, as each approach clarifies different aspects of texture perception and structural behavior. Samples produced at higher pH were perceived as softer (Fig. 3d). This finding aligns with the close relationship between WHC and perceived hardness described by

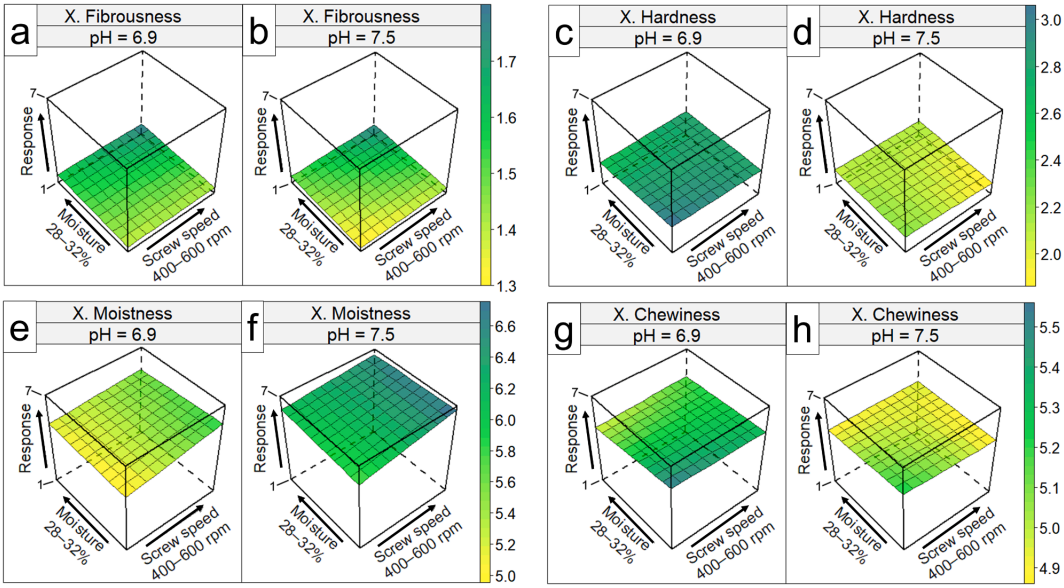

Fig. 3. Response surface plots depicting the variations of sensory properties of the extrudates produced with different extrusion parameters: fibrousness (a, b), hardness (c, d), moistness (e, f), and chewiness (g, h). X.—indicates the sensory texture.

Muhialdin and Ubbink (2023). In our study, moistness was also significantly affected by pH and strongly correlated with WHC of whole extrudates (Pearson's $r = 0.88$). The similarity between the response surface plots of WHC and moistness (Figs. 1c,d and Fig. 3e,f) confirms that WHC of the whole extrudate, rather than milled, is a reliable predictor of juiciness in TVP matrices. We can conclude that the lower hardness of the samples at higher pH reflects the enhanced water retention, which softened the structure during mastication. This effect is technologically relevant, as juiciness and tenderness are among the most desired textural traits in meat analogs (Starowicz et al., 2022). Moreover, many plant-based systems require dedicated water-binding agents to achieve them (De Angelis et al., 2024c).

The model coefficients for chewiness did not significantly influence this sensory parameter, although the model showed an $R^2 = 0.75$. The surface model indicated lower chewiness at high pH (Fig. 3h), although this parameter was not significant ($p > 0.05$). Chewiness is defined as the energy required to chew TVP and is related to hardness and moistness attributes, making it complex and difficult to evaluate (De Angelis et al., 2020; Kaleda et al., 2021). No differences were observed in the other attributes (Supplementary material, Table S3), including bitterness and astringency, which are common undesirable attributes in plant-based alternative products.

3.2.4. Comparison with reference samples

To evaluate the flexibility of the extrusion conditions for producing DWMP-based TVP, two reference extrudates were produced as controls. PPI is one of the main protein sources used in different plant-based alternative products on the market (Sha & Xiong, 2020), and for this reason was extruded alone as the first reference. The second reference was produced by mixing the PPI with PSC, because legume proteins are typically mixed with polysaccharides due to their thickening effect and contribution to the formation of a fibrous texture (Sha & Xiong, 2020).

The comparisons between the sample TVP and the reference TVP are reported in Table 4. The experimental design results highlighted that the modulation of the process conditions leads to the development of products with highly variable properties. These products, despite the

Table 4
Comparison of the properties of extruded texturized vegetable proteins prepared from the three raw materials.

Trial	WHC whole (g H_2O/g extrudate)	WSI whole (%)	Hardness (g)	Springiness (%)	X.Fibrousness (-)	X.Moistness (-)	X.Hardness (-)	X.Chewiness (-)
Reference PPI	1.93 ± 0.10	12.64 ± 0.42	4880 ± 352	36.42 ± 4.69	1.28 ± 1.02	4.17 ± 1.04	3.78 ± 0.81	5.11 ± 0.83* **
Reference PPI_PSC	3.16 ± 0.15	11.85 ± 0.45	2319 ± 475	41.49 ± 9.09	2.72 ± 0.75	6.00 ± 1.03	2.11 ± 0.76	4.94 ± 0.80* **
S1	2.03 ± 0.04*	12.80 ± 0.54*	3917 ± 552	50.54 ± 4.60**	1.39 ± 1.04*	4.78 ± 0.94	3.11 ± 0.76*	5.56 ± 0.62* **
S2	3.18 ± 0.02**	11.43 ± 0.12**	2371 ± 666**	54.66 ± 9.36	1.44 ± 0.70*	6.00 ± 0.84	2.83 ± 0.79**	5.33 ± 0.69* **
S3	2.36 ± 0.08	13.02 ± 0.14*	4568 ± 482*	39.29 ± 5.64* **	1.56 ± 0.86*	5.33 ± 0.91**	2.56 ± 0.78**	4.89 ± 0.76* **
S4	2.32 ± 0.08	13.64 ± 0.20	3353 ± 342	43.16 ± 5.05* **	1.83 ± 0.86*	5.44 ± 0.98	2.89 ± 0.68**	5.22 ± 0.73* **
S5	2.47 ± 0.06	14.41 ± 0.34	4251 ± 389*	44.66 ± 7.45* **	1.28 ± 0.75*	5.89 ± 0.90**	2.22 ± 0.65**	5.17 ± 0.71* **
S6	4.38 ± 0.17	11.05 ± 0.45**	2483 ± 413**	49.36 ± 6.83**	1.44 ± 0.62*	6.56 ± 0.92**	1.89 ± 0.58**	4.89 ± 0.76* **
S7_1	3.36 ± 0.05**	14.88 ± 0.22	5049 ± 1060*	44.63 ± 5.60* **	1.44 ± 0.92*	6.17 ± 0.86**	1.94 ± 0.54**	5.00 ± 0.69* **
S7_2	3.23 ± 0.11**	15.25 ± 0.38	4827 ± 785*	47.33 ± 5.22**	1.56 ± 0.86*	5.94 ± 0.80**	2.33 ± 0.77**	4.78 ± 0.55* **
S8	3.56 ± 0.19	11.26 ± 0.41**	2846 ± 316**	45.80 ± 6.09**	1.78 ± 0.81*	6.83 ± 0.51**	2.06 ± 0.73	4.94 ± 0.73* **

* the value is similar to the reference PPI; ** the value is similar to the reference PPI_PSC according to Dunnett's test ($p > 0.05$). PPI—pea protein isolate; PPI_PSC—mix with pea protein isolate and pea starch concentrate; samples from S1 to S8 were produced with PPI_DWMP— mix with pea protein isolate and durum wheat meal protein (75:25 w:w). WHC—water holding capacity, and WSI—water solubility index. X.—indicates the sensory evaluation of textural attributes.

utilization of innovative raw materials, could match the functional characteristics of extrudates produced using conventional sources like PPI and PPI_PSC. For example, in the WHC of whole extrudates conditions set to pH 6.9, 28% of moisture, and 600 rpm screw speed (trial S2) resulted in a WHC similar to the PPI_PSC reference extrudate, but when the screw speed was lowered to 400 rpm (trial S1) the result was similar to the PPI reference. At pH 7.5, a WHC similar to PPI_PSC could be obtained at 32% moisture and 400 rpm screw speed instead (trial S7). Lowering the screw speed could be considered an important aspect in terms of energy consumption, because the SME increased by increasing the screw speed value (Table 3). SME is indicative of the overall mechanical treatment intensity occurring during extrusion and can be used to compare and optimize the processes (De Angelis et al., 2020). In the WSI of whole extrudates all the samples extruded at 600 rpm were comparable to the reference PPI_PSC, whereas the samples extruded at pH 6.9 and 400 rpm were similar to the reference PPI.

Hardness is usually a critical parameter for TVP. In fact, texture modulation is one of the challenges addressed by changing processing conditions and formulation (De Angelis et al., 2024c; Opaluwa et al., 2025). As reported in Table 4, the TVP produced at 600 rpm (S2, S6, and S8) showed similarities with the PPI_PSC reference, whereas the samples produced at 400 rpm (S3, S5, and S7) were similar to the PPI reference. Springiness value was mostly comparable to the PPI_PSC reference, but some samples were also comparable to the PPI reference. Specifically, at pH 6.9 the moisture content needed to be at 32% to reach a similar springiness value of the PPI reference. The fibrousness of all experimental TVP samples matched the PPI reference, while hardness and moistness values aligned more closely with the PPI_PSC benchmark. Chewiness was comparable to both references across all trials.

These results demonstrate that DWMP, used here as an innovative upcycled ingredient, can be effectively incorporated into TVP formulations without compromising fibrous structure formation or negatively affecting key sensory attributes. It can also produce different textures by adjusting the processing conditions.

4. Conclusions

In this study, we evaluated the effects of screw speed, feed moisture, and pH on the properties of TVPs, highlighting the most influential factors. Interestingly, the effect of pH under low-moisture conditions was previously unexplored, providing important information for further research. Screw speed had a significant influence on WHC, WSI, specific volume, and hardness. The increased expansion associated with higher screw speed enhanced WHC while reducing both WSI and instrumental hardness. By contrast, increasing feed moisture decreased WHC and increased instrumental hardness and springiness while promoting the formation of a more fibrous and anisotropic structure. Adjusting the pH to 7.5 enhanced WHC and the sensory perception of moistness, while reducing sensory hardness. Textural attributes were primarily governed by screw speed and moisture content. Higher screw speed reduced hardness and increased specific volume, whereas higher moisture content enhanced network density, increased hardness, and promoted the formation of fibrous structures.

The findings of this study demonstrate that controlling screw speed, moisture content, and pH enables targeted modulation of TVP structure and sensory profile. DWMP proved compatible with pea protein in low-moisture extrusion, and under appropriate processing conditions, produced extrudates with functional and sensory characteristics comparable to established commercial formulations. This highlights its potential for valorization in sustainable plant-based meat analog applications. This study provides a practical framework for semolina producers seeking to valorize milling side-streams and for extrusion technologists aiming to refine processing strategies for TVP production.

Ethical statement

This study was conducted in accordance with the World Medical Association Declaration of Helsinki and the Principles of Academic Ethics by Tallinn University of Technology (TalTech, 2017), ensuring the safety of research and data processing. This study does not involve collecting sensitive personal data. Nevertheless, responsible data management was implemented to prevent participant identification and ensure confidentiality, as per the General Data Protection Regulation (GDPR). Participants were informed of the general purpose of the study and provided informed consent prior to taking part. All the participants were informed that participation was voluntary, allowing them to withdraw from the study at any time.

Funding

This research was supported by: 1) the Ministerial Decree no. 351 of 9th April 2022, based on the PNRR - funded by the European Union - NextGenerationEU - Mission 4 “Education and Research”, Component 1 “Strengthening the offer of education services: from nurseries to universities”- Investment 4.1 “Extension of the number of research doctorates and innovative doctorates for public administration and cultural heritage” grant number H91I22000970007; 2) the Agritech National Research Center and funding from the European Union NextGenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)–MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 June 17, 2022, CN00000022). This manuscript reflects only the authors' views and opinions; neither the European Union nor the European Commission can be considered responsible for them.

CRediT authorship contribution statement

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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.

Acknowledgments

We thank Carmine Summo, our beloved friend and esteemed colleague, for his important contribution to the manuscript. Although he is no longer with us, his dedication continues to inspire us. The authors would like to thank Casillo Next Gen Food s.r.l. (Corato, Italy) for the experimental support and for kindly providing the dry-fractionated proteins of defatted durum wheat meal.

We acknowledge Karel Talvistu (TFTAK) for helping with the extrusion experiments, Maarja Maasikmets (TFTAK) for organizing the sensory analyses, and Helen Vaikma (TFTAK) for sensory protocol development.

Supplementary materials

Supplementary material associated with this article can be found, in

the online version, at doi:10.1016/j.afres.2026.101925.

Data availability

Data will be made available on request.

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ISSN 2585-6901 (PDF)
ISBN 978-9916-80-517-6 (PDF)