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INNOVATIVE TECHNOLOGICAL APPLICATIONS OF PLANT-BASED INGREDIENTS

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Al professor Summo

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Summary (English)

Concerns regarding climate change and the increasing of the world population have grown the demand for sustainable, healthier, and ethical food products. As a consequence, the market of animal-based food shifts towards plant-based products. The substitution of meat involves using plant protein ingredients, and among the crops, legumes have been identified as one of the most promising protein sources. Wet fractionation is the most commonly applied technology for obtaining protein and starch ingredients. Compared to the wet process, dry fractionation is a more efficient alternative that saves chemicals, energy and water. Indeed, dry fractionation involves a combination of milling and dry separation, through air classification or tribo-electric separation of starch and proteins. Dry-fractionated ingredients are characterized by lower purity than wet-fractionated ones, since dry fractionation allows to separate a fine fraction richer in proteins from a coarse fraction enriched in starch. The latter is often considered a co-product of protein production, destined to feed industry. Being a solvent-free technology, dry fractionation can retain the native structure and functionality of macromolecules, making it advantageous for tailoring the ingredients in food development.

In this doctoral thesis the technological properties of dry-fractionated ingredients and their applications in innovative food products were explored. Those activities are related to the mission M2C1 of Italian PNRR "Sustainable agriculture and circular economy" and the 12 Goal established by the 2030 United Nations' Agenda "Responsible consumption and production".

Activities begin with an in-depth study of techno-functional properties of dry-fractionated ingredients. Information was collected from the available literature and converted into an overview of the best application in food products, based on the properties of the dry fractionated ingredients (Chapter 1). Indeed, dry-fractionated ingredients have some peculiar properties linked to their native preserved structure that could help in tailoring food products. Then, in order to clearly establish a difference between dry and wet fractionated proteins, a comparison of their techno-functional, rheological and chemical properties was carried out (Chapter 2). A collection of 17 protein ingredients from dry (n=9) and wet (n=8) fractionation was analysed. Dry-fractionated ingredients, due to their native structure and the presence of other non-protein components, appeared to be suitable in foods in which foaming, solubility and emulsion capacity are required.

Those properties were explored in real, innovative food products, specifically a sponge cake with a dry-fractionated egg-replacer (Chapter 3), which was submitted to rheological, textural and sensory characterization. The main objective was to explore the suitability of dry-fractionated protein-rich fractions from lentil, chickpea and mung bean to be used as egg replacer. The effect of species and concentration on cake properties was evaluated. The higher content of dry fractionated ingredients simulated egg behavior, resulting in a volume and thickness similar to the sponge cake made from eggs. Sensory properties revealed that chickpea and lentil proteins had less impact on sensory properties, while dry-fractionated mung bean produced a sponge cake with a strong legume flavor.

Dry fractionation technology could also be suitable for valorizing food by-products, such as the defatted cake generated during the extraction of the oily fraction from durum wheat (*Triticum turgidum* L. var. *durum*) bran and germ. Indeed dry-fractionated durum wheat cake protein was explored to produce a snack bar rich in protein and fibers (Chapter 4). The experimental design helped to choose the best formulation for the snack bar, achieving the desired nutritional profile while maintaining good textural and sensory properties. Chapter 5 describes the use of the same by-product, in combination with pea protein isolate, in the production of texturized vegetable protein (TVP) using a low-moisture extrusion-cooking process. TVPs are generally applied in meat alternative

products. The concentrations of dry-fractionated durum wheat cake protein and pea protein isolate were varied, and the techno-functional, textural, volatile and sensory properties of TVPs were evaluated. The results showed that the formulation containing at least 30% of durum wheat cake protein had similar texture compared to the control produced with the 100% pea protein isolate, and had increased fibrousness, which is an important parameter in meat alternative products. Moreover, no off-odor and off-flavors were observed in all the samples under investigation, highlighting the potential application of this by-product.

To complete these studies, considering that the dry-fractionated starch-rich fraction is still underutilized, its use in plant-based jelly candies has been explored, taking advantage of the gelling ability of starch (Chapter 6). A micro a macro structure characterization was carried out in order to better understand the differences with commercial corn starch and dry-fractionated pea starch. The results indicated that the jelly candies produced with dry-fractionated pea starch had a gumminess similar to the control jelly candy produced with gelatine, despite the fragile structure of the gel formed. Moreover, jelly candies with dry-fractionated pea starch presented an intense color, which could be beneficial in reducing food colorants in those kinds of products.

Riassunto

Le preoccupazioni per il cambiamento climatico e l'aumento della popolazione mondiale hanno causato l'aumento della domanda di prodotti alimentari sostenibili, sani ed etici. Di conseguenza, il mercato degli alimenti di origine animale ha subito una transizione verso prodotti di origine vegetale. La sostituzione della carne comporta l'uso di ingredienti proteici di origine vegetale e, tra le varie colture disponibili, i legumi sono stati identificati come una delle fonti proteiche più promettenti. L'estrazione a umido è la tecnologia più comunemente applicata a diverse colture vegetali, per ottenere ingredienti quali proteine e amido mediante l'impiego di sostanze chimiche, energia e acqua. Tuttavia, il frazionamento secco rimane un'alternativa più efficiente dal punto di vista dell'impiego delle risorse. Infatti, il frazionamento a secco prevede di estrarre l'ingrediente tramite una combinazione di macinazione e di separazione fisica, mediante un classificatore ad aria o un separatore triboelettrico dell'amido e delle proteine. Gli ingredienti frazionati a secco sono caratterizzati da minore purezza rispetto a quelli estratti con la tecnologia a umido, poiché la tecnologia di produzione è basata sulla sola separazione di una frazione fine arricchita in proteine da una grossolana arricchita in amido, quest'ultima spesso considerata un co-prodotto della produzione proteica e destinata all'industria mangimistica. Essendo una tecnologia priva di solventi, il frazionamento secco può mantenere la struttura e le funzionalità delle macromolecole native, che possono essere pienamente sfruttate nella formulazione di prodotti alimentari.

In questa tesi di dottorato sono state studiate le proprietà tecnologiche degli ingredienti frazionati a secco e le loro applicazioni in prodotti alimentari innovativi. Queste attività sono legate alla missione M2C1 del PNRR "Agricoltura sostenibile ed economia circolare" e all'Obiettivo 12 stabilito dall'Agenda 2030 "Consumo e produzione responsabili".

Le attività hanno previsto una prima parte di ricerca bibliografica sugli ingredienti frazionati a secco e sulle loro proprietà tecnologiche e funzionali. Per questa attività sono state raccolte informazioni in letteratura, successivamente convertite in una sintesi in cui sono state descritte le proprietà degli ingredienti frazionati a secco e la loro possibile applicazione in prodotti alimentari (Capitolo 1). Infatti, gli ingredienti frazionati a secco hanno alcune proprietà peculiari, legate alla loro struttura nativa, che potrebbero essere sfruttate nella produzione di prodotti alimentari. Per stabilire le differenze tra le proteine frazionate a secco ed estratte a umido è stato quindi effettuato un confronto delle loro proprietà tecnologiche, funzionali, reologiche e chimiche (Capitolo 2). Sono stati analizzati 17 ingredienti proteici ottenuti mediante frazionamento secco (n=9) e umido (n=8), in cui è stato dimostrato che le proteine estratte ad umido presentano differenti proprietà che dipendono dalla tecnologia di estrazione. Gli ingredienti frazionati a secco presentavano una struttura nativa e altre componenti non proteiche che potevano essere vantaggiose in alimenti in cui sono richieste capacità schiumogene, emulsionanti e solubilità.

Queste proprietà sono state studiate in prodotti alimentari innovativi, in particolare è stato prodotto un pan di spagna con un sostituto dell'uovo prodotto con proteine frazionate a secco (Capitolo 3), seguito da una caratterizzazione reologica, strutturale e sensoriale. L'obiettivo principale era quello di osservare le differenze negli impasti prodotti a partire da lenticchia, cece e fagiolo mungo, impiegati come sostituto dell'uovo, in base alla specie e alla loro concentrazione. Il contenuto più elevato di ingredienti frazionati a secco simulava il comportamento delle uova, ottenendo un volume e uno spessore simili a quelli del pan di spagna prodotto con le uova. Le proprietà sensoriali hanno rivelato che le proteine di cece e lenticchia hanno avuto un impatto minore sulle proprietà sensoriali, mentre il fagiolo mungo ha prodotto un pan di spagna dal forte sapore di legumi.

La tecnologia di frazionamento a secco potrebbe anche essere adatta per la valorizzazione di sottoprodotti alimentari, quali il panello residuo dell'estrazione olearia dal mix di crusca e germe derivanti dalla macinazione del grano duro. Infatti, le proteine del panello disoleato di grano duro, frazionate a secco, sono state impiegate per produrre una barretta ricca in proteine e fiber (Capitolo 4). L'impiego del disegno sperimentale ha aiutato a scegliere la migliore formulazione della snack bar. Nel capitolo 5 lo stesso sottoprodotto è stato utilizzato, in combinazione con l'isolato proteico di pisello, per la produzione di proteine vegetali testurizzate (TVP) ottenute mediante la tecnologia di estrusione-cottura a bassa umidità. Le TVP sono generalmente impiegate in prodotti alternativi alla carne. Il sottoprodotto, frazionato a secco, e l'isolato proteico di pisello sono stati utilizzati in diverse concentrazioni, e sono state valutate le proprietà tecnologiche, funzionali, strutturali, i composti volatili e le proprietà sensoriali delle proteine testurizzate corrispondenti alle varie miscele. I risultati hanno mostrato che la formulazione contenente almeno il 30% di panello di grano duro frazionato a secco aveva una durezza simile al controllo prodotto con il 100% di isolato proteico di pisello e presentava una maggiore fibrosità, che è un parametro importante nei prodotti alternativi alla carne. Inoltre, in tutti i campioni esaminati non sono stati osservati odori e sapori sgradevoli, evidenziando la potenziale applicazione di questo sottoprodotto.

L'amido frazionato a secco spesso è un co-prodotto della produzione di proteine ed è stato studiato per la sua capacità gelificante in gelatine alla frutta plant-based (Capitolo 6). È stata effettuata una caratterizzazione micro e macro-strutturale al fine di comprendere meglio le differenze con un amido di mais commerciale e amido di pisello frazionato a secco. I risultati hanno indicato che le gelatine alla frutta prodotte con amido di piselli frazionato a secco avevano una gommosità simile a quella delle caramelle gommose prodotte con gelatina, nonostante il gel formato dallo stesso fosse fragile. Inoltre, le caramelle gommose con amido di piselli frazionato a secco presentavano un colore intenso, utile per ridurre i coloranti alimentari presenti in questa tipologia di prodotto.

General introduction

1. Plant-based ingredients

Plant-based diet is becoming increasingly recurring because it is considered healthier and more sustainable compared to the omnivorous one (Aschemann-Witzel et al., 2021; Kim et al., 2018; Satija and Hu, 2018; Pais et al., 2022). Cereals and pseudocereals are recognized as a good nutritional source characterized by presence of complex carbohydrates, proteins, fats, vitamins and minerals, playing an important role in human nutrition (Senarathna, Mel, and Malalgoda, 2024). Among the crops, oat, wheat, rice and barley are the most popular, usually utilized in many food applications (Bartłomiej, Justyna, and Ewa, 2012; Senarathna, Mel, and Malalgoda, 2024).

Legumes are high-protein crops recognized as suitable options to produce plant-based foods (Aschemann-Witzel et al., 2021). Their production involves little quantity of resources; therefore, they are environmentally sustainable (Affrifah, Uebersax, and Amin, 2023). Legumes are crops largely cultivated worldwide and are one of the most ancient foods known, constituting an essential dietary supplementary (Maphosa and Jideani, 2017; Flint-Hamilton, 1999). Indeed, the ancient Romans used to cultivate various legume species, including beans, lentils and chickpeas, which were a staple food in their diet (Carlini, Lucini, and Velázquez, 2024). Together with cereal grains, legumes are well-known in the Mediterranean diet, whose benefits are recognized both in literature (Tosti, Bertozzi, and Fontana, 2018; Serra-Majem et al., 2019; Guasch-Ferré, and Willett, 2021) and by UNESCO as an intangible cultural heritage (Serra-Majem et al., 2019). Besides being rich in proteins, that represent from 20% to 35% of the seed, legumes are a good source of nutrients, such as fiber, carbohydrates, vitamins and minerals (e.g., iron, zinc, magnesium) (Polak et al., 2015; Gu et al., 2023). The protein content and the nutritional profile vary significantly within the species and the cultivation area (Gu et al., 2023). Moreover, the bioactive compounds in the seeds have numerous health benefits including chronic disorders, diabetes, cancer, cardiovascular diseases (Serra-Majem et al., 2019; Clemente, and Jimenez-Lopez, 2020). However, the concurrent presence of antinutritional compounds often limits the use of legumes in foods. Among them, phytates, generally found in many plant-based foods, have two functions. First, they hinder the enzymes of protein degradation in the small intestine and stomach, and second, they chelate minerals (Samtiya, Aluko, and Dhewa, 2020). Indeed, since phytates are negatively charged structures, they bind positively charged minerals such as zinc, iron, magnesium and calcium, forming complex, thus, reducing the bioavailability of those ions and causing lower absorption (Samtiya, Aluko, and Dhewa, 2020). Tannins are phenolic compounds that could affect protein digestibility due the formation of complex with proteins, causing the inactivation of digestive enzymes (Serrano et al., 2009). Enzyme inhibitors, of which the most known is trypsin inhibitor, are responsible for the decreasing of protein digestion, amino acid availability and pancreatic hyperplasia (Samtiya, Aluko, and Dhewa, 2020). Due to these undesired effects, many research articles in the scientific literature are focused on reducing the content of antinutrients in legume-based foods. However, the health benefits of legumes and their chemical composition make them still preferred for plant-based ingredient production, especially for starch and protein extraction.

2. Plant-based protein and starch extraction

Plant-based ingredients are useful in both traditional and innovative food applications. Each ingredient has its own functionality which depends on the raw material used, but also on the extraction method (Li et al., 2024). Plant-based powder ingredients are commercially available in form of starch and protein. In order to obtain them, the raw material may undergo physical pre-treatments such as defatting and dehulling process, which could improve the raw material characteristics (Pua et al., 2022; Ravindran, Singh, and Singha, 2024; Fernando, 2021). For example, dehulling could remove some antinutritional factors, and affects some physical properties (Li et al., 2024; Fernando, 2021). Then legumes could be processed through different methods, including wet and dry fractionation.

2.2. Wet fractionation

Wet fractionation is a wide technology used to extract protein and starch from plants. In protein extraction the raw material is first milled and then the flour is dispersed in water to create a suspension (slurry) to reach the disentanglement of the tissue structures (Schutyser and Van der Goot, 2011). Then, the pH is adjusted to alkaline or acid conditions, followed by an iso-electric precipitation or ultrafiltration and spray drying to obtain a powder ingredient (Schutyser and Van der Goot, 2011). The conditions used for the wet fractionation process are often not specified in literature (Fernando, 2021), leading to a difference in the protein functional properties in the same species. The advantage of the wet fractionated ingredients is linked to their high protein content (around 85-90%) (Li et al., 2024; Schutyser and Van der Goot, 2011). However, this process requires the intensive use of resources such as water, energy and chemicals and generates acidic effluents containing proteinaceous material (Assatory et al., 2019). For example, Berghout, Boom, and Van der Goot (2014) stated that to produce 1 kg of lupin protein isolate, more than 80 kg of water were needed, 22.4 kg of hexane, 40 g of NaOH and approximately 40 g of HCl. Therefore, the use of resources has been one of the most critical aspects that is induced to search for a more sustainable technology. Moreover, the need of varying pH to obtain the disentanglement of individual components, coupled with the adoption of high temperatures to dry the ingredient into powder, lead to the unfolding of the protein fraction, negatively influencing their functionality (Schutyser and Van der Goot, 2011). Indeed, those harsh conditions make proteins lose their native structure and deeply influence the degree of denaturation and their aggregation (Fernando, 2021).

For starch extraction the process is quite similar, but it requires one step more. In fact, it has been reported that to extract corn starch, sulfur dioxide (SO_2) or bisulfite (HSO_3^-) is used for more than 20 h at 48–52 °C to make the kernels become softer, through the disruption of the protein matrix that covers the starch granules. This step is one more step involving chemicals (Assatory et al., 2019; Ji, Seetharaman, and White, 2004), but it is necessary to make the separation between proteins and starch bodies easier (Ji, Seetharaman, and White, 2004). From the technological point of view, the use of high temperatures, in order to dry the material into powder, could decrease the starch quality (Assatory et al., 2019).

2.3. Dry fractionation

Dry fractionation is based on the physical separation of starch from proteins. In this technology, the raw material is first milled to obtain a fine flour, that is necessary to disrupt the endosperm or cotyledon into pieces, without harshly damaging starch granules and other tissue components (Fernando, 2021; Pelgrom, Boom, and Schutyser, 2015). In this first phase the particle size plays a pivotal role in having a successful separation between starch and proteins (Pelgrom, Boom, and Schutyser, 2015). The efficiency of milling depends on different factors i) the fat concentration, that can soften the material, involving more milling energy, ii) moisture content which could cause adhesion between protein and starch, causing a difference in the milling quality (Fernando, 2021).

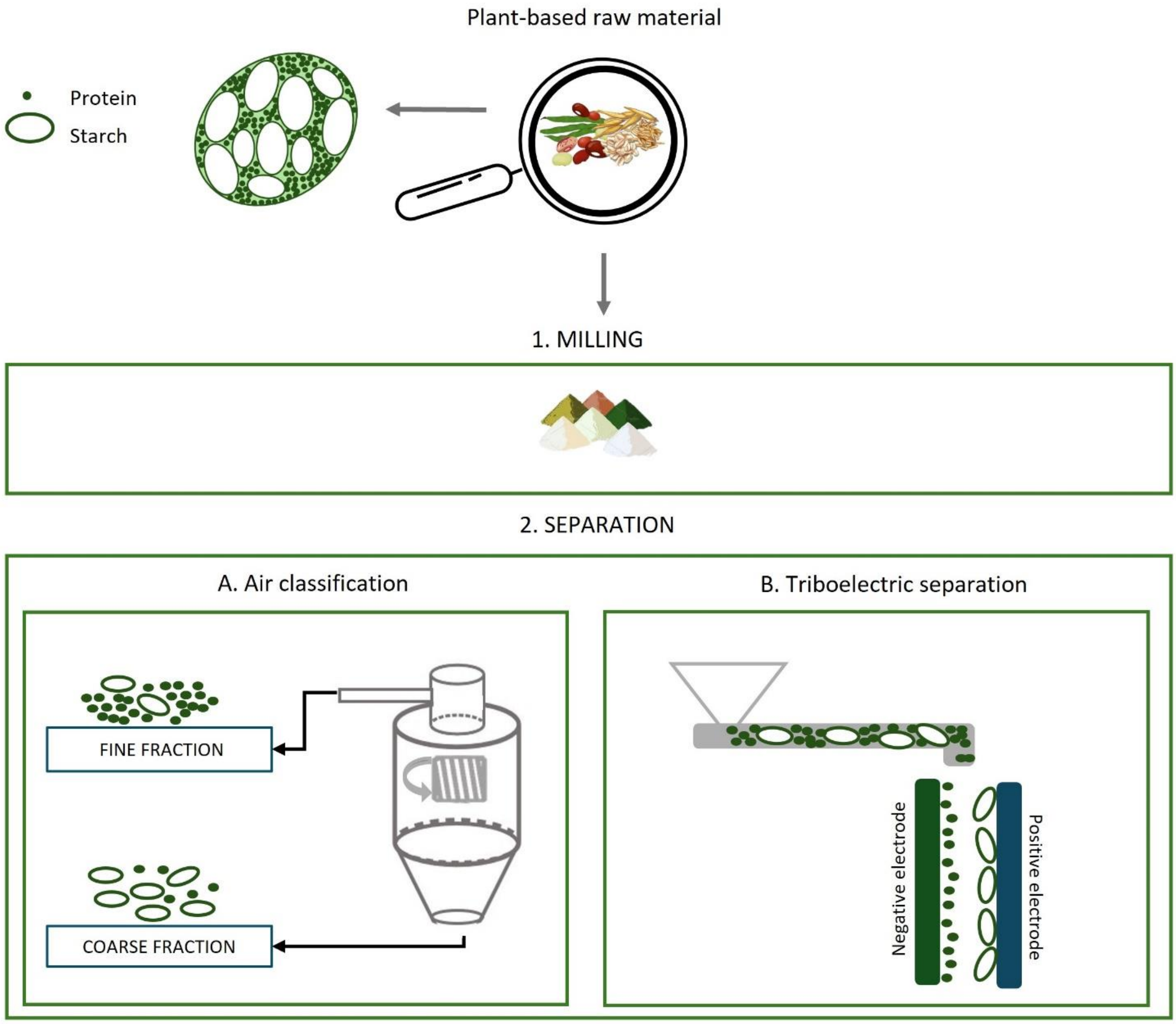

Figure 1. Dry fractionation of plant-based ingredients. Elaboration from Schutyser et al. (2025).

The flour particles after milling should be sufficiently small and disintegrated to be separated. However, excessive milling could lead to an inefficient separation due to the high starch damage that, in turn, causes the aggregation of the cell components that do not separate (Fernando, 2021; Pelgrom et al., 2014; Pelgrom et al., 2013). After milling, the raw material undergoes a separation process which is commonly made via air classification (Figure 2A). A classifier wheel, rotating at high speed, detaches the protein bodies (light and small fine fraction, around 1-3 µm) from starch granules (heavy and large coarse fraction, around 20 µm) according to their aerodynamic properties that depend on density, shape and particle size (Fernando, 2021; Schutyser and Van der Goot, 2011; Pelgrom et al., 2013). The efficiency of the air classification process depends on both the milled material characteristics and the classifier wheel speed settings (Li et al., 2024). Another way to separate proteins from starch is by sieving, which separate particles based on difference in size (Fernando, 2021; Tabtabaei et al., 2016). However, both air classification and sieving are based, partially or totally, on size differences and this constitutes a limitation in terms of purity of the material. Indeed, some starch and protein particles may enter in the “wrong” fraction as a result of the particle-particle cohesion, therefore the air turbulence could not separate similar particle size/density particles (Fernando, 2021; Tabtabaei et al., 2016). One alternative to air classification is tribo-electric separation (Figure 2B), that is based on the separation of tribo-charged particles under the influence of an external electric field (Tabtabaei et al., 2016). The detachment between the two fractions happens when two surfaces are in contact with each other, transferring the electrons from the surface of one material to the other, making the separation more efficient than air-classification and sieving (Fernando, 2021).

The advantage of dry fractionation is liked to different aspects. First, this process is more sustainable compared to the wet fractionation process thanks to the reduced or no use of water. Further, it does not need a stabilization process after separation, like heating or drying, and it is easily scalable (Fernando, 2021). Compared to wet fractionated ingredients, dry fractionated ones have lower purity, meaning that the protein or starch enrichment is minor compared to the wet fractionated ones. The purity of the ingredient depends on the physiochemical characteristics of the seed (Pulivarthi et al., 2023). However, the presence of other components, such as starch, in the protein-rich fraction may not represent a problem in some food applications (Schutyser and Van der Goot, 2011). On the other hand, in dry-fractionated protein ingredients, the lack of chemicals during concentration preserves the native structure of the protein, thus preserving its functionality in tailoring food products.

Therefore, due to its sustainable characteristics, dry fractionated ingredients were explored in this thesis, aiming to the production of sustainable plant-based ingredients.

2.3.1 Dry fractionation applied to plant-based ingredients

Dry fractionation technology could be applied to different crops, including cereals, pseudocereals and legumes. Since the legumes are protein-rich food, the extraction of proteins from those crops becomes advantageous obtaining high protein material. Indeed, Schutyser et al. (2015) reported that from legume crops it could be reached a protein content around 49-70 g protein/100 g dry matter, according to the raw material characteristics. Legume crops, compared to cereals, give higher protein purity due to the higher protein content and to the greater size and uniformity of their starch granules (Schutyser et al., 2015). However, some legume crops are rich in fats, which could obstacle the dry fractionation process. For example, different authors (Schutyser et al., 2015; Schutyser et al., 2025) reported that the dry fractionation of soy and chickpea is difficult due to their high lipid content, therefore one way to process them is removing oil before milling, possibly with sustainable technologies like supercritical fluid extraction (Schutyser et al., 2025; Pulivarthi et al., 2023). Since dry fractionation method is a technology aimed at helping the transition toward a green and environmentally friendly process, it could be applied also to food by-products or waste streams as raw material (Pulivarthi et al., 2023).

Part of this thesis addresses the plant-based ingredients differences according to the extraction method and to their application in innovative food, with a focus on the dry fractionated ingredients. The second part will be dedicated to the application of the dry-fractionated ingredients in innovative food products with an emphasis on legumes and durum wheat by-products, the latter being the defatted cake generated during the extraction of the oily fraction from durum wheat (*Triticum turgidum* L. var. *durum*) bran and germ.

2.4. Thesis scope and aims

This research project and PhD thesis aimed to study plant-based ingredients, their properties and functionality to specifically apply them in innovative food products, pursuing the 12 Goal established by the 2030 United Nation Agenda “Responsible consumption and production” (FAO, 2025) and the mission M2C1 of PNRR “Sustainable agriculture and circular economy” (Ministero dell’Ambiente e della Sicurezza Energetica, 2025). Figure 1 is a visual representation of the content of this doctoral thesis.

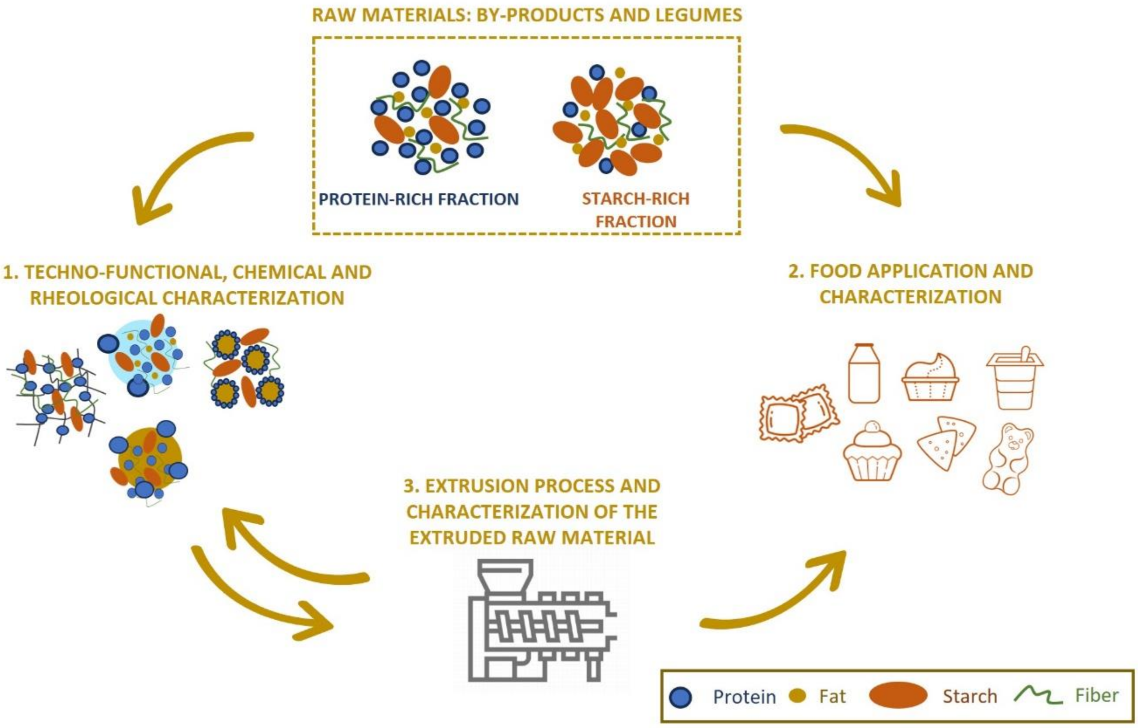

Figure 2. Overview of the thesis content.

First, in order to pursue those aims, bibliographic research regarding the techno-functional properties of plant-based ingredients obtained through dry fractionation technology was accomplished. This activity was a starting point to assess which functionality and food applications were already studied in literature and what was lacking (**Article no. 1**). In addition, a comprehensive characterization of 17 plant-based ingredients wet and dry extracted was conducted to understand their techno-functional, rheological, and chemical properties and their possible application in food products (**Article no. 2**). This screening was needed to have some key attributes and properties of the raw materials.

Then, the application of plant protein and starch ingredients from legumes and durum wheat cake was carried out in different innovative food products. Lentils, mung bean and chickpea dry-fractionated proteins were exploited to produce dispersion, used as an egg replacer, in sponge cake batter (**Article no. 3**). Specifically, mung bean was chosen due to its nutritional properties, but also lentils and chickpea were considered because they are typical of the Mediterranean basin, where the study was carried out.

Durum wheat cake is a by-product of durum wheat supply chain, which is defatted and rich in protein and fibers. Therefore, through dry fractionation, it could be recovered to produce a high protein ingredient, useful to enhance the protein and fiber content in food products. Dry-fractionated durum wheat cake was used to produce a snack bar, with enhanced protein and fiber content (**Article no. 4**). The same by-product was also applied in texturized vegetable proteins (TVPs) which are commonly used in developing meat alternatives (**Article no. 5**). The main objective of this research was maximizing its content in the TVP formulation. This activity was carried out in collaboration with TFTAK AS (Tallinn, Estonia).

Starch ingredients obtained from dry fractionation are primarily destined for the feed industry, but they have peculiar functional properties that could be used in food products. Indeed, dry fractionated pea starch was applied to produce jelly candies, due to its gelling property. This research aimed to develop an innovative product by exploiting the possibility of valorizing the starch-rich fraction (**Article no. 6**).

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List of publications

De Angelis, D., **Latrofa, V.**, Caponio, F., Pasqualone, A., & Summo, C. (2024). Techno-functional properties of dry-fractionated plant-based proteins and application in food product development: A review. *Journal of the Science of Food and Agriculture*, *104*(4), 1884-1896.

De Angelis, D., **Latrofa, V.**, Squeo, G., Pasqualone, A., & Summo, C. (2024). Techno-functional, rheological, and chemical properties of plant-based protein ingredients obtained with dry fractionation and wet extraction. *Current Research in Food Science*, *9*, 100906.

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Chapter 1. Techno-functional properties of dry fractionated plant-based proteins and application in food product development

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Abstract

Dry-fractionated protein concentrates are gaining attention because they are produced using versatile and sustainable technology, which can be applied to a wide range of plant material. To facilitate their utilization in new product development, it is crucial to obtain a comprehensive overview of their techno-functional properties. The present review aims to examine the techno-functional properties of dry-fractionated protein concentrates and describe their primary applications in food products, considering the published works in the last decade. The techno-functional properties of proteins, including water absorption capacity, emulsifying and foaming properties, gelling ability or protein solubility, are relevant factors to consider during food formulation. However, these properties are significantly influenced by the extraction technology, the type of protein and its characteristics. Overall, dry-fractionated proteins are characterized by high protein solubility, high foaming ability and foam stability, and high gelling ability. Such properties have been exploited in the development of food, such as bakery products and pasta, with the aim of increasing the protein content and enhancing the nutritional value. Additionally, innovative foods with distinctive textural and nutritional characteristics, such as meat and dairy analogues, have been developed by using dry-fractionated proteins. The results indicate that the study of these ingredients still needs to be improved, including their application with a broader range of plant materials. Nevertheless, this review could represent an initial step to obtaining an overview of the techno-functional properties of dry-fractionated proteins, facilitating their use in foods.

Keywords: plant-based protein; food product development; techno-functional properties; gelling ability; protein solubility; water absorption.

Introduction

The interests and the investments in the alternative protein sector are growing as a response to the needs of producing food in a more sustainable, healthier and ethical way, aiming to satisfy the growing population, as well as the consumer's demand (Ma et al., 2022; Otero et al., 2022). Indeed, the market size for protein ingredients is expected to grow at a yearly rate of 5.8% from 2023 to 2030 (Protein Ingredients Market Size, Share & Trends Report). Plant-based sources of protein include pulses, cereals, pseudocereals and nuts, whereas innovative protein sources are insects, fungi, microorganisms and microalgae (Otero et al., 2022). Plant-based ingredients are marketed in the form of protein concentrates, isolates and flours, and they are used to improve the nutritional composition of different foods (e.g. bread, bakery goods and pasta) (Ma et al., 2022; Otero et al., 2022; Schutyser et al., 2015) as well as to produce alternatives to the animal-based food products (e.g.meat and dairy analogues) (Ma et al., 2022; Boukid et al., 2022).

One of the most diffused methods to produce protein ingredients is the wet extraction, also called wet fractionation, which is generally carried out by an alkaline extraction followed by an isoelectric precipitation, with a final drying stage. This process is able to produce protein isolates with a very high protein content, usually higher than 80% (Ma et al., 2022; Boukid et al., 2022; Lie-Piang et., 2023). However, wet extraction requires a large amount of resources in terms of chemicals, water and energy, contrasting with the purpose of promoting a sustainable food system (Schutyser et al., 2015; Lie-Piang et., 2023). A comprehensive description of the methodologies and of the different technologies for wet extraction is provided elsewhere (Ma et al., 2022; Lie-Piang et., 2023; Boukid et al., 2021).

A dry fractionation (DF) approach is largely more sustainable than wet extraction processes (Lie-Piang et., 2023) because it consists of physical methods of concentration of the protein. In brief, DF operates on the premise that it can effectively separate protein bodies from other non-protein components, such as starch granules and fibers, utilizing suitable milling technologies, including pin and jet milling (Schutyser et al., 2015; Fernando et al., 2021; Assatory et al., 2019). Subsequently, the milled flour undergoes separation into two fractions: one enriched in starch and the other in protein, achieved through physical separation methods. The most commonly employed technique for DF is air classification. This method involves the introduction of air currents into a classifier chamber, generating centrifugal and gravitational forces within the chamber that fractionated the flour into fine and coarse particles distinguished by the differences in size and density (Assatory et al., 2019). Electrostatic separation is another DF technology, traditionally used in the mineral industry but recently implemented in the food sector (Fernando et al., 2021). This method relies on tribo-electric charging and, consequently, on the surface properties of flour particles (Tabtabaei et al., 2019). It involves the transfer of the electrons from the surface of one material to another, taking advantage on the fact that proteins tend to be charged to a much higher extent than carbohydrates as a result of the presence of ionizable groups in their amino acid residues (Assatory et al., 2019). Notably, electrostatic separation offers advantages when the aim is to fractionate dietary fibers (Wang et al., 2016; Zhang et al., 2019). A comprehensive description of DF technology is available in recent review articles (Schutyser et al., 2015; Fernando et al., 2021; Assatory et al., 2019).

An important aspect to consider is related to the mass yield of the two processes. Although wet extraction generally results in a protein content higher than 70%, the mass yield of the protein ingredient can be quite low. Indeed, there is an inverse relationship between the protein content of the fraction and the mass yield (Schutyser et al., 2015). Dumoulin et al. (2021) reported that the mass yield of faba bean protein was 8% when a wet extraction process was employed (61% protein content), whereas it increased to 34% with the use of a DF process (protein content 64%). However, the yields are highly dependent on the extraction conditions and on the species (Malik and Singh, 2022). The processing of green and yellow peas using an industrial air classification system produced a protein fraction with a yield of 35–44% and a protein content of 46–57% (Rempel, Geng and Zhang, 2019). De Angelis et al. (2021a) processed green pea, yellow lentil and red lentil using a pilot plant and obtained a mean yield in the range 14.8–23.8% and a protein content of 61.9% and 53.9%, respectively. Finally, DF requires a quantity of water 5.5-fold lower than wet extraction technology (Dumoulin et al., 2021).

From a practical point of view, to facilitate the utilization of dry-fractionated proteins in the food industry and guarantee their proper exploitation during the food design, it is fundamental to have a clear overview of their functional and technological properties. For example, properties such as water absorption capacity, gelling ability or protein solubility are relevant factors to be considered during the food formulation, but they are greatly influenced by the type of protein and by the extraction technology. In particular, such information on dry-fractionated protein is still not comprehensively documented in scientific literature. For this reason, the aim of this review is to discuss the functional properties of the protein concentrates produced by DF and to disclose the main food applications in which they are used as ingredients.

Current trends and raw materials for dry fractionation

This review is based on a literature search carried out using the scientific databases Scopus, Web of Science Core Collection and Google Scholar, considering all the works published in a 10-year range from 2013 to present (1st June 2023). Different keywords were employed for the search, including, but not limited to: 'dry fractionation', 'air classification', 'functional properties', 'technological properties', 'dry-fractionated protein', 'meat analogues', 'cheese analogues', 'bakery products', 'dairy analogues' and their combinations using Boolean operators. The search within title, abstract and keyword led to a total of 873 results, which were refined considering only the articles belonging to the Agricultural and Biological Sciences, Chemical Engineering, and Multidisciplinary categories. The research produced 89 articles, composed of 16 reviews and 73 research papers studying the DF process, of which 23 articles were focused on the food applications. The results of the literature research were also analyzed using VOSViewer 1.6.19 (https://www.vosviewer.com; Centre for Science and Technology Studies, Leiden University, The Netherlands). In particular, the analysis of keywords co-occurrence was carried out, with a minimum requirement of two occurrences for each keyword.

The results of the bibliographic search show an increasing trend in the number of publications in the last 10 years (Fig. 1a). In detail, it is interesting to note that the studies regarding the application of dry-fractionated proteins in food are very recent, with references recorded only since 2018 (Fig. 1a) but are rapidly increasing also due to the request of plant-based foods by vegetarian, vegan and flexitarian consumers. The studies regarding the DF process and the functional properties of the obtained proteins, instead, showing an increase from 2013 to 2018, are now numerically more stable. This was also evident in the network map of keyword co-occurrences depicted in Fig. 2, showing the keywords used in the studies colored according to the year. By visualizing the relationships between keywords, this map provides a clear overview of the thematic evolution over time.

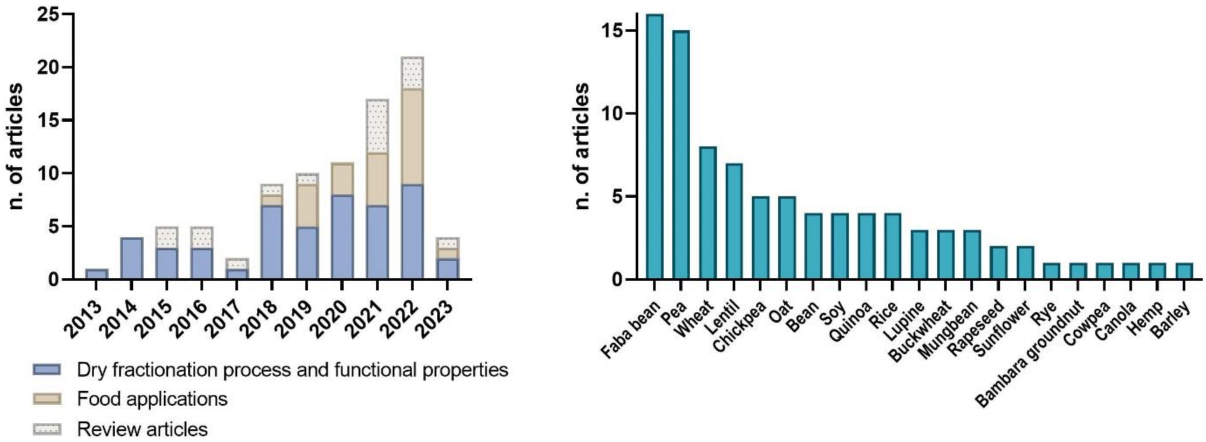

Figure 1. Number of published articles (a) and plant sources studied in the last 10 years (b) according to the literature search.

The keywords related to the study of the fractionation process, such as ‘milling', ‘air classification’, ‘processing', ‘pulses’ and ‘functionality’, are characteristic of the early period considered in this literature review. By contrast, starting from 2020 to the present, the keywords have shifted towards dealing with the physicochemical properties of proteins and their applications in innovative foods, including keywords such as ‘meat analogues’ and ‘gelled emulsion’. This reflects the evolving interests and a growing emphasis on applications and properties beyond the DF process.

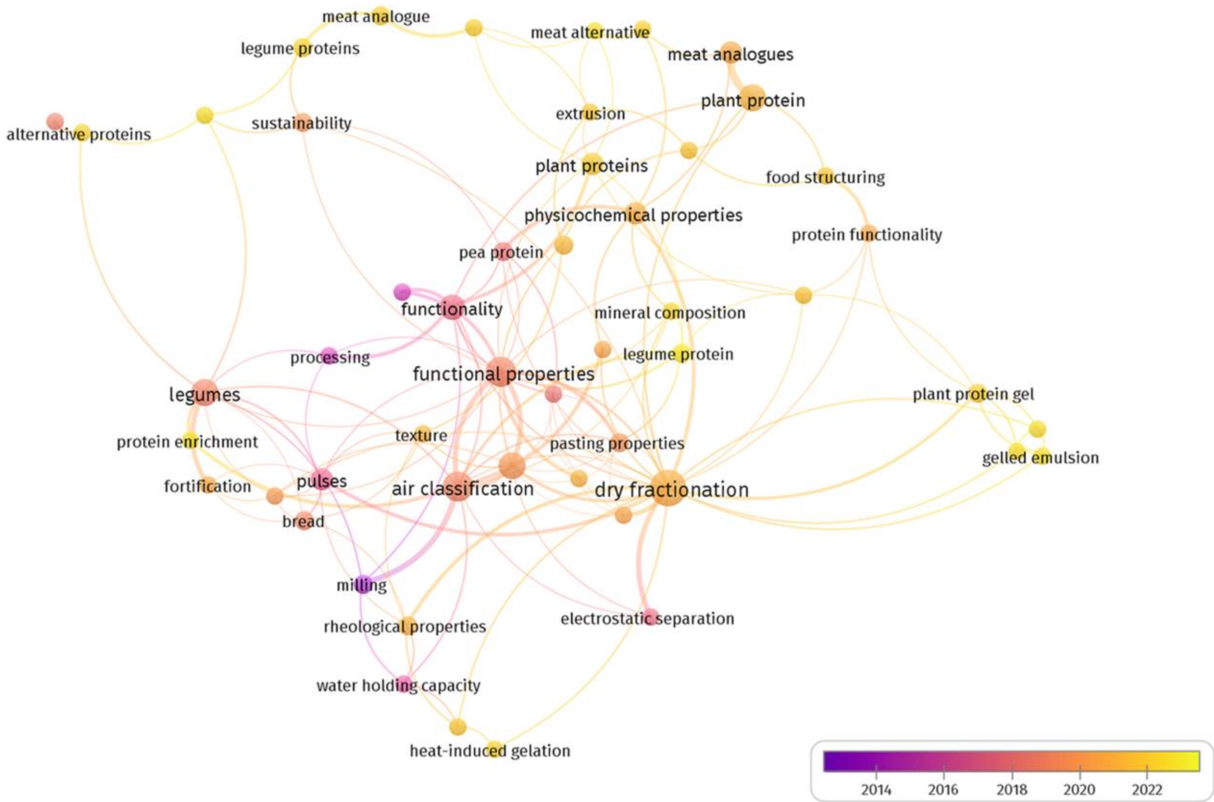

Figure 2. Network map of keyword co-occurrences colored according to year.

The plant sources object of the DF are prevalently pea and faba bean, followed by wheat and lentil (Fig. 1b). This may be related to the good attitude of such species to being dry-fractionated proteins as a result of the presence of large starch granules, high initial protein content and low lipid content (Assatory et al., 2019; De Angelis et al., 2021b; Schutyser and Van der Goot, 2011). Despite the lower number of studies concerning the other leguminous, cereal and pseudocereal crops, a total of 21 different crops have been studied, confirming the versatility of the DF process and its potential in supporting the valorization of the minor crops. Indeed, DF can be easily applied to different legume and cereal crops (Assatory et al., 2019) to obtain highly added value ingredients, with the exception of raw material having an oil content higher than 35% (Lie-Piang et., 2023). From this perspective, DF can not only be used to produce ingredients from the major crops but also might be a solution to exploit and valorize even the neglected and underutilized grains, facing problems related to the loss of genetic resources and of biodiversity heritage. Neglected and minor grains are well adapted to local environments, require low input for their growth and tolerate stress in a way that they can be grown in marginal areas (Desire et al., 2021). As described elsewhere, underutilized grains include, for example, varieties of cowpea, groundnuts, sesame, African locust bean and lima bean (Desire et al., 2021), and the Apulian black chickpea (Summo et al., 2019). This is a relevant and modern objective of the Italian scientific community, which aims at the development of marginal areas to promote multifunctional production systems enhancing agroecological and socio-economic sustainability (https://agritechcenter.it). Moreover, this would also promote protein diversification and increase the self-sufficiency of protein production (Ferreira et al., 2021).

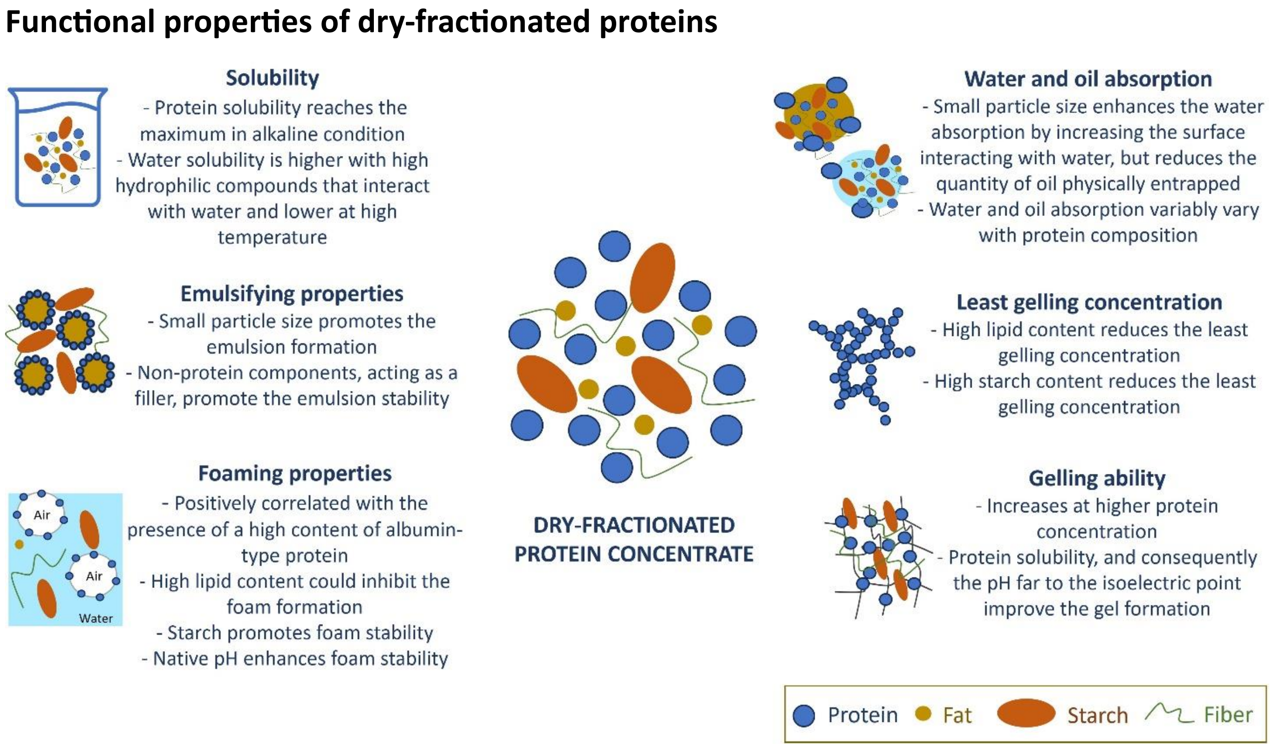

Figure 3. Highlights of the characteristics of the tecno-functional properties of dry-fractionated proteins.

Protein solubility, foaming ability, emulsifying capacity and liquid binding capacity are the main surface properties of protein ingredients, whereas hydrodynamic properties are all those related to viscosity, gelation and pasting ability (Yada, 2017). Both surface and hydrodynamic properties, affected by the protein extraction technology, the botanical species considered and the protein concentration level (Ma et al., 2022), influence the food applications of the fractionated proteins. The main findings related to the functional properties of different botanical species subjected to DF are summarized in Fig. 3 and Table 1 and are discussed in the following sections.

Table 1. Summary of the raw materials and of the techno-functional properties of the studies under investigation.

Year	Reference	Raw material	Concentration range	Techno-functional Properties
2020	Diedericks et al.	Bambara groundnut black-eye variety (*Vigna subterranea* L.)	29-33%	Minimum gelling concentration:11-12% (w/w) Rheological properties
2018	Silventoinen et al.	Industrial barley endosperm fraction	26-28%	PS (pH 11): 91.9 % FC (at native pH, 5.1): 48 % FS (at native pH, 5.1): 48 % WAC (at native pH, 5.1): 1.23 g/g OAC (at native pH, 5.1): 1.13 g/g LGC (at native pH, 5.1): 10 %

2023	de Paiva Gouvêa et al.	Commercial pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) protein concentrates	50-58%	EA: ~ 14.03 – 18.00 m^2/g ES: ~ 15 min FC: ~85- 97.8% FS after 60 min: 65% - 95 % WAC: ~ 1.34 - 2.50 g water/g OAC: ~ 1.5 - 2.84 g oil/g LGC: 10 - 12%
2020	Vogelsang-O'Dwyer et al.	Faba bean (*Vicia faba* L. cv. Imposa)	64%	PS (pH 8): 90% FC: ~ 25 – 55 % FS: ~ 85 % OAC: 124 % Minimum gelling concentration: 7% Rheological properties
2022	Nasrollahzadeh et al.	Two commercial Hemp (*Cannabis sativa* L.) protein concentrates	56–61%	PS and Pasting properties WAC: 0.8 - 1.3 g water/g LGC: 18-26 %
2022	Funke et al. (a)	Green lentils (*Lens culinaris* Medik. variety Richlea)	42-54%	PS (pH 7): 62-64% Emulsion and interfacial properties e.g., ζ-potentials, particle size distribution
2022	Funke et al. (b)	Brown lentils (*Lens culinaris* Medik. variety “Alb Leisa – Die Kleine”)	16–59%	Emulsion properties: e.g., average diameters (d32) and ζ-potentials storage stability, particle size distribution
2021	De Angelis et al. (b)	Red and yellow lentils (*Lens culinaris* Medik.), green pea (*Pisum sativum* L.), kabuli chickpea (*Cicer arietinum* L.)	47-57%	WAI: 3.48 – 3.98 g/g WSI: 21.38 – 27.30 % WAC: 0.42 – 0.89 g water/g OAC: 0.45 – 0.53 g oil/g
2014	Pelgrom et al.	Lupine (*Lupinus angustifolius* L.)	54-59%	Foaming properties Viscosity
2022	Schlangen et al.	Dehulled mung beans (*Vigna radiata*), dehulled yellow peas (*Pisum sativum* L.), whole cowpeas (*Vigna unguiculata* (L.) Walp.)	42-58%	WAC: 1.53 to 2.14 g water/g LGC: ~ 8 – 10% Rheological properties
2022	Yang et al.	Mung bean (*Vigna radiata* (L.) R. Wilczek)	23-24%	Rheological properties
2019	Tabtabaei et al.	Pin-milled navy bean flour (*Phaseolus vulgaris* L.)	34-38%	EAI: 59.00 % ES: 106.60 % FC: 88.00 %

				FS after 120 min: 41.5b% WAC: 131.80 % OAC: 82.4
2021	De Angelis et al. (a)	Green pea (*Pisum sativum* L.), yellow and red lentil (*Lens culinaris* Medik.)	54-62%	WAI: 3.91 - 4.04 g/g WSI: 24.56 – 26.98 % WAC: 0.80 g water/g OAC: 0.49 – 0.51 g oil/g
2020	Saldanha do Carmo et al.	Yellow peas (*Pisum sativum* L. var. Ingrid), faba beans (*Vicia faba* L. var. Kontu)	44-61%	PS (pH 9): 23-80% EC: 6/10 g protein/g oil EAI: 12.32 – 18.80 m^2/g ESI: 12.22 – 14.24 min FC: 26.56 – 54.84 % WAC: 0.58 – 0.97 g water/g OAC: 1.11 – 1.14 g oil/g Gelling properties
2015	Pelgrom et al.	Yellow peas (*Pisum sativum* L.)	43%	Rheological properties
2013	Pelgrom et al.	Yellow peas (*Pisum sativum* L.)	51-55%	WAC: ~ 3.5 (w/w)
2020	Solaesa et al.	Quinoa cv. Titicaca (*Chenopodium quinoa* Willd.)	13-24 %	WAI: ~ 2 – 6.5 g/g WSI: ~ 8 – 17.5 % EA: ~50-55% FC: 7 ml FS: 56 % WHC: 2.31 g/g WAC: 0.97 g/g LGC: 12 % Rheological and pasting properties
2018	Opazo-Navarrete et al.	Quinoa sweet varieties Atlas and Riobamba (*Chenopodium quinoa* Willd.)	32-33%	WAC and Solubility at different temperatures Pasting properties
2016	Wang et al.	Brown rice (*Oryza sativa* L.) bran	13-17%	WAC: 4.7 g/g OAC:6.7 g/g
2019	Lee et al.	Germinated brown rice variety Chuchung	8%	WSI: 6.23 % WAI: 2.23 g/g OAC: 92.93 %

				Pasting properties
2019	Silventoinen et al.	Rice bran (*Oryza sativa* L. ssp. Indica)	20-27%	PS (pH 8): ~80%
2021	Silventoinen et al.	Commercial wheat and rye bran	31%	PS (pH 8): 43.6 - 75.50 % WAC: 1.20 g water/g OAC: ~ 1 g oil/g Pasting properties

PS = Protein solubility WAI = Water absorption index; WSI = Water solubility index; WAC = Water absorption capacity; WHC = Water holding capacity determined after 24h; OAC = Oil absorption capacity; EA= Emulsion activity, calculated by measuring the volumes of the emulsified layer after centrifugation; ES = emulsion stability, determined after heating and centrifugation; EAI = Emulsion activity index evaluated using the spectrophotometric method; ESI = Emulsion stability index, evaluated using the spectrophotometric method; EC= Emulsion capacity evaluated using the conductimetry; FC = Foaming capacity; FS = Foaming stability; LGC = Least gelling concentration.

Surface properties: solubility, emulsifying and foaming ability

Protein solubility specifically measures the amount of protein that remains soluble in water, and it is generally determined at different pH. Protein solubility reaches the maximum in alkaline conditions (Ma et al., 2022; Vogelsang-O'Dwyer et al., 2020; Silventoinen et al., 2021). In particular, Saldanha do Carmo et al. (2020) investigated the protein solubility of air-classified pea and faba bean proteins at different pH and reported a two-fold higher solubility in faba bean compared to pea protein at pH 10, with values ranging from 51% to 67% and from 23% to 40%, respectively. Regarding the cereals, dry-fractionated wheat and rye bran showed a protein solubility of 75.5% and 43.6% at pH 8, respectively (Silventoinen et al., 2021), whereas dry-fractionated barley protein showed only 22.2% protein solubility at pH 8, which reached 91.9% at pH 11 (Silventoinen et al., 2018). By contrast, superior solubility of dry-fractionated navy bean concentrate compared to the isolated one was reported in Tabtabaei et al. (2019) with the maximum value found at pH 8. These results confirm great variability depending not only on the pH, but also on the protein composition and, consequently, on the botanical species (Saldanha do Carmo et al., 2020).

Protein extraction technology significantly influences protein solubility. Vogelsang-O'Dwyer et al. (2020) found a higher solubility in the dry-fractionated faba bean compared to the same protein extracted with alkaline extraction and isoelectric precipitation. The processing conditions (e.g. alkaline/acidic environment and heating) occurring during the wet extraction had a negative effect on the solubility and, indeed, may lead to denaturation, causing an exposure of the hydrophobic regions, resulting in higher surface hydrophobicity (Vogelsang-O'Dwyer et al., 2020).

By contrast to protein solubility, the water solubility index (WSI) defines the percentage of the total soluble solids that remain in the aqueous phase after a heating process (De Angelis et al., 2021b; Solaesa et al., 2020). Therefore, WSI includes not only protein, but also all the hydrophilic compounds that interact with water (Saldanha do Carmo et al., 2020; Solaesa et al., 2020; Du et al., 2014). In particular, soluble protein, starch and fibers contribute to the WSI, indicating an influence of the chemical composition (De Angelis et al., 2021b; Solaesa et al., 2020) DF affects the WSI, such that this property is generally higher in the protein fraction compared to the starch-rich fraction, as reported for yellow and red lentils, green pea and kabuli chickpea (De Angelis et al., 2021b), quinoa

(Solaesa et al., 2020), and brown rice (Lee et al. 2019). It should be also considered that the temperature of analysis significantly influences WSI values (e.g. increasing the temperature from 60 °C to 95 °C led to a reduction of WSI from ~17.5% to ~8% in the fine fraction of quinoa) (Solaesa et al., 2020). A high solubility might be exploited for all the food applications requiring protein solubilization, such as non-acid protein beverages and milk substitutes (Fig. 4).

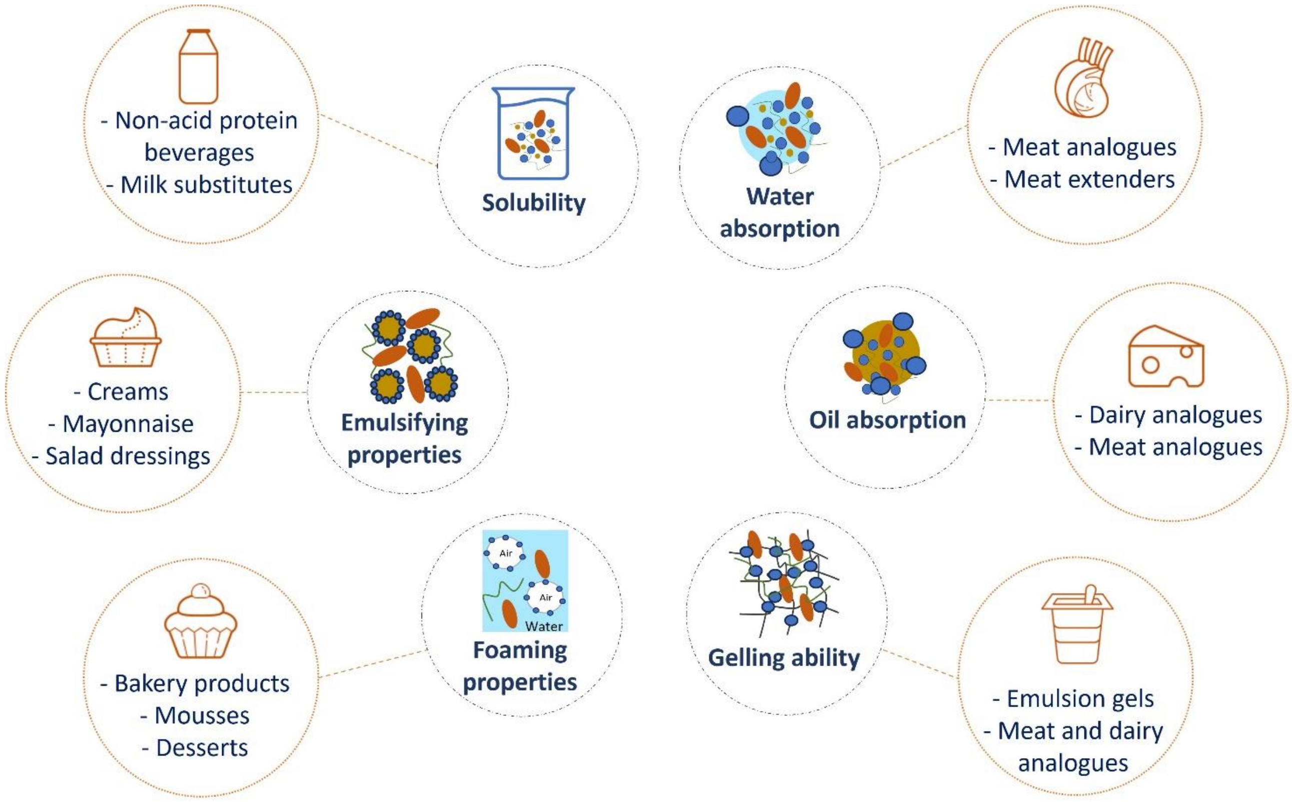

Figure 4. Food applications of the dry-fractionated proteins suggested for the high values of the main techno-functional properties.

Emulsifying properties are linked to the ability of proteins to build a layer around oil droplets that are dispersed in a water phase. These properties depend on different physicochemical factors including surface hydrophobicity, solubility and particle size (Ma et al., 2022; Saldanha do Carmo et al., 2020; Boye et al., 2010b). Emulsifying properties can be assessed through several methods. The emulsion activity index (EAI) involves measuring the turbidity of an emulsion using a spectrophotometric method. Then, the stability of the emulsion can be also determined by evaluating the emulsion stability index (ESI). Emulsion activity (EA) is determined by measuring the volume of the emulsified layer after a centrifugation of the emulsion, while the emulsion stability (ES) is a measurement of the stability of the emulsion over a certain time, and it is usually assessed after heating and a centrifuging the emulsion (Boye et al., 2010a). Another method, emulsion capacity (EC), quantifies the amount of oil required to switch from an oil-in-water emulsion to a water-in-oil emulsion and is usually determined using a conductivity meter.

Silventoinen et al. (2021) prepared stable emulsions using dry-fractionated wheat and rye brans, noting that the presence of non-protein components such as dietary fibers may have a positive role in emulsion stabilization by increasing their viscosity in the continuous aqueous phase. Moreover, Solaesa et al. (2020) reported a 62.5% emulsifying activity in quinoa fine fraction, and the emulsifying activity showed no differences regardless of whether it was determined at 25 °C and

80 °C, indicating a good stability of the emulsion. Solaesa et al. (2020) also suggested a significant influence of the particle size of the fine fraction (D_{50}, 171 µm) with respect to enhancing the accessibility of protein to the oil–water interface compared to the coarse fraction, which had a D_{50} of 1220 µm. Therefore, finer particle size can enhance the emulsifying properties. Funke *et al*. (2022a; 2022b) suggested that dry-fractionated lentil protein has a good potential in stabilizing an emulsion, corroborating the findings of Tabtabaei et al. (2019) who revealed a similar emulsifying ability comparing navy bean dry-fractionated protein and commercial soy protein concentrate, and better foaming stability compared to the soy. Saldanha do Carmo et al. (2020) found a higher emulsifying activity in pea protein fractions compared to faba bean. In general, the good emulsifying properties of dry-fractionated proteins make them suitable for emulsified foods, such as creams, mayonnaise and salad dressings.

The foaming properties of dry-fractionated proteins have been assessed in different studies, which often highlight a high ability to form the foam and long persistence of the foam over time. As reported by Vogelsang-O'Dwyer et al. (2020) and by Tabtabaei et al. (2019) the DF process is able to preserve the native structure of proteins, leading to higher foaming ability and stability compared to the protein extracted using a wet fractionation protocol. The foaming properties of dry-fractionated protein may be positively correlated with the presence of a higher content of albumin-type protein compared to the wet-extracted proteins (Tabtabaei et al., 2019; Yang et al., 2022a). Indeed, during the isoelectric precipitation, only the globulin fractions are recovered, whereas the majority of the albumin fraction is lost (Lie-Piang et., 2023; Vogelsang-O'Dwyer et al., 2020). Albumins are superior for foaming because they form very strong interfacial films (Lie-Piang et., 2023). The foaming ability is also dependent on the botanical species; for example, using the same DF conditions, Saldanha do Carmo et al. (2020) reported a higher foaming capacity in dry-fractionated pea protein compared to faba bean protein, with mean values of approximately 55% *versus* 45%, respectively. Moreover, a non-significant effect of the dehulling procedure on the foaming properties was also highlighted. It should be also considered that the foaming properties are affected by the presence of other components. For example, a high lipid content (e.g. 11% dry matter) may inhibit the ability of the protein to form and stabilize the foam (Solaesa et al., 2020), whereas the presence of starch has been reported to have a positive impact on foaming properties because of its ability to stabilize the foam (Silventoinen et al., 2018). Furthermore, other factors including the protein concentration (Ma et al., 2022) and pH (Silventoinen et al., 2018) influence the foaming properties. In barley, a pH far from the isoelectric point (i.e. below 4 and above 8) promotes foam capacity, whereas native pH (5.1) showed the best foam stability because of the protein neutral net charge that increases protein–protein interaction (Silventoinen et al., 2018).

For this reason, Ma *et al*. (2022) highlighted the importance of standardizing the protocols to determine the functional properties of the ingredients, aiming to achieve an easier evaluation of their technological performances. Overall, the good foaming properties of dry-fractionated protein can be exploited for the development of food requiring the incorporation of air in the matrix, such as mousses, desserts and some bakery products (Yang et al., 2022b).

The water and oil absorption capacities (WAC and OAC) of proteins measure the amount of water or oil they can hold per unit mass, respectively (Ma et al., 2022). WAC is also called water holding capacity (WHC) or water binding capacity, and as reviewed by Ma *et al*. (2022) the water absorption can be either determined at room temperature or after a thermal treatment with a variable temperature. In the latter case, the analysis named water absorption index (WAI) is often used. When determined after a heating process, the water absorption reflects the ability of the protein to bind water by forming a gel, and it depends on the particle size and on the hydrophilic groups present in the fraction (Solaesa et al., 2020; Lee et al., 2019) and it also may be related to the presence of damaged starch in the protein fraction (De Angelis et al., 2021b). A higher WAC was recorded in the hemp fractions with a lower granulometry because a smaller particle size increases the specific area that interacts with water (Nasrollahzadeh et al., 2022). Moreover, other hydrophilic constituents besides proteins, such as fibers and polysaccharides, may have an influence on the water absorption (De Angelis et al., 2021b; Saldanha do Carmo et al., 2020). Schlangen et al. (2022) reported that the WAC of dry-fractionated mung bean, yellow pea and cowpea proteins was 2.15, 1.53 and 2.06 g/g, respectively, and could be linked to the different protein characteristics, rather than to the protein concentration. Instead, De Angelis et al. (2021b) reported WAC values in the range 0.42–0.89 g/water g in dry-fractionated lentils, chickpea and pea proteins, which increased to 2.98–3.48 g/water g when the capacity to bind water was determined after a heating treatment (i.e. WAI) in the same species. Overall, the dry-fractionated proteins show a lower ability to bind water than the proteins extracted with the wet fractionation technologies (Vogelsang-O'Dwyer et al., 2020) as a result of protein denaturation. As previously discussed, the alkaline/acidic treatment, as well as the drying thermal process, leads to exposure of the hydrophobic groups, causing a reduction of the solubility and consequently an increase in the WAC (Vogelsang-O'Dwyer et al., 2020). Investigating the WAC is important for setting up the processing of the ingredients. For example, WAC helps to understand the amount of water needed for the extrusion process to produce plant-based meat analogues.

The oil binding capacity, also called the oil absorption capacity (OAC), fat binding capacity or fat absorption capacity, is usually less variable than the WAC because it shows values near 1 g of oil absorbed per gram of protein. In particular, previous studies report 1.24 g/g in faba bean (Vogelsang-O'Dwyer et al., 2020), 1.13 g/g in barley fine fraction (Silventoinen et al., 2018), 1.0 g/g in wheat bran (Silventoinen et al., 2021) and 0.82–0.91 g/g in navy bean (Tabtabaei et al., 2019), whereas De Angelis et al. (2021b) reported a range of 0.45–0.53 g/g for dry-fractionated lentils, chickpea and pea proteins. The particle size could have an influence on the OAC because smaller particles may reduce the quantity of oil that can be physically entrapped by the protein powder (Silventoinen et al., 2021). A good OAC could be useful in dairy and plant-based meat analogues; by contrast, a low OAC could prevent the absorption of oil in fried foods (Vogelsang-O'Dwyer et al., 2020; de Paiva Gouvêa et al., 2023). Moreover, the determination of WAC and OAC is required to appropriately design the food process and/or the formulations because these properties allow a clear understanding of the quantity of liquid phases that the protein ingredient can hold. For example, high WAC (e.g. > 2 g/g) and high OAC (e.g. > 1 g/g) could be useful to enhance the cooking yield or prevent the cooking loss. However, it should be considered that comparison data among studies is difficult because they depend on the methodology used for the determination (Ma et al., 2022). This leads to concerns related to poor standardization, which makes any comparison of the results difficult, highlighting the need for more standardized methods.

Gelling ability and rheological properties of dry-fractionated proteins

Gelation is a process by which a system goes from a liquid to a solid-like state characterized by a three-dimensional matrix in which the liquid phase is retained. Proteins form a heat-set gel (Saldanha do Carmo et al., 2020; Nasrollahzadeh et al., 2022; Schlangen et al., 2022); therefore, a heating step followed by cooling is necessary for the gel formation. In particular, proteins need to be denatured and unfolded to expose the functional groups responsible for the aggregation reactions and the formation of a network able to retain water molecules. The mechanisms of protein gelation are comprehensively described elsewhere (Schlangen et al., 2022; Pelgrom et al., 2015). The gelling properties of proteins can be assessed by determining the least (or minimum) gelling concentration

(LGC) (Ma et al., 2022). However, rheological evaluations carried out by an oscillatory rheometer (Vogelsang-O'Dwyer et al., 2020; Solaesa et al., 2020; Schlangen et al., 2022) or by a rapid visco amilograph (Silventoinen et al., 2021; Silventoinen et al., 2018; Nasrollahzadeh et al., 2022) can give a more detailed information about the gelation phenomena and the physicochemical quality of the gel. Overall, dry-fractionated proteins show good gelling properties. As evaluated by Diedericks et al. (2021) the LGC of Bambara groundnut protein-enriched flour fractions was 11–12% (w/w), whereas mung bean, yellow pea and cowpea are characterized by a LGC near 10% (Nasrollahzadeh et al., 2022). de Paiva Gouvêa et al. (2023) reported a LGC of 10% and 12% for pea and faba bean protein concentrates obtained by air classification, respectively. A slightly higher value was found in dry-fractionated quinoa protein, which showed a LGC in the range 12–14% (Solaesa et al., 2020). Proteins have better gel ability compared to the other components (e.g. starch) (Nasrollahzadeh et al., 2022), thus, LGC is negatively influenced by lipids and by starch. The latter competes for water during gelation (Solaesa et al., 2020).

Interestingly, Vogelsang-O'Dwyer et al. (2020) investigated the gelling properties of dry-fractionated and isolated (wet-extracted) faba bean proteins, highlighting how the alkaline extraction and isoelectric precipitation, led to a reduction of the gelling ability. Vogelsang-O'Dwyer et al. (2020) reported a LGC of 7% and 12% for the dry-fractionated and wet-extracted proteins, respectively, together with a higher G' modulus evaluated by the rheological analysis in the dry-fractionated proteins, meaning that the latter formed a stronger gel. Nasrollahzadeh et al. (2022) who confirmed that the LGC was affected by the extraction technology and ingredient composition, reported an 18–26% LGC for dry-fractionated hemp and 12–26% for hemp extracted with aqueous extraction and isoelectric precipitation.

It was recently described how extensive wet fractionation may cause a reduction of the gelling ability of the proteins as a result of a combination of factors, including the isoelectric precipitation, amount of sugars, drying procedures and differences in ash content (Kornet et al., 2021). Moreover, dry-fractionated proteins containing high protein content (e.g. solution with 15% of protein) have better gelling ability (i.e. they form stronger gels), suggesting that not only the protein composition, but also the protein characteristics and concentration have an influence on such properties (Nasrollahzadeh et al., 2022). Other factors affecting the gel formation include pH and consequently the protein solubility, as discussed by Silventoinen et al. (2018) who found that the LGC of dry-fractionated barley protein was lower at pH 7 and 8 compared to pH 3 and 5 (near the isoelectric point of the protein).

Overall, the gelling properties of dry-fractionated proteins may be exploited for the preparation of food characterized by a solid-like structure, such as dairy products, emulsion gels and creams, yogurt, and meat analogues.

Food application of dry-fractionated proteins

The studies aimed at using dry-fractionated proteins in food applications focus on: (i) food supplemented with proteins (which are not the main ingredient) with the aim of improving the nutritional value and (ii) innovative foods with peculiar textural and nutritional characteristics, prepared with the proteins as main ingredient. In the first case, conventional foods (e.g. bread, bakery products and pasta) are the object of the formulation, with a total of 11 published studies. By contrast, plant-based meat analogues and dairy alternatives are included in the second case, for a total of 12 research articles (Table 2).

Table 2. Food products partially supplemented or totally prepared using the dry-fractionated ingredients, with the indication of plant species, protein content (if applicable) and amount added.

Year	Reference	Product	Plant species	Dry-fractionated ingredient	Protein content of the dry-fractionated ingredient	Amount added
2023	De Angelis et al.	Gluten-free Focaccia flat bread	Pea (*Pisum sativum* L.)	Commercial protein concentrate	55%	5%
2022	Gangola et al.	Crackers	Faba bean (*Vicia faba* L.)	Commercial protein concentrate	~60%	40%
2021	Xing et al.	Bread	Chickpea (*Cicer arietinum* L.)	Dry-fractionated chickpea and its sourdough	31%	20-30%
2020	Hoehnel et al.	Bread	Faba bean (*Vicia faba* L.)	Commercial protein concentrate combined with other protein ingredients	61%	5.72%
2020	Xing et al.	Sourdough	Kabuli chickpea (*Cicer arietinum* L.)	Protein concentrate	~40%	12:6 g:g (protein: water)
2019	Zhang et al.	Bread	Wheat bran	Two batches of wheat bran	15-16%	2-10%
2019	Hoehnel et al.	Bread	Faba bean (*Vicia faba* L.)	Commercial protein concentrate and other protein ingredients	61%	15%
2018	Ficco et al.	Bread	Durum and soft wheat	Purple-pericarp and blue-aleurone	n. a.	30-40%
2022	Hoehnel et al.	Fresh high-protein hybrid pasta	Buckwheat and Faba bean (*Vicia faba* L.)	Dry-fractionated proteins	23-61%	4-13%
2021	Gangola et al.	Pasta	Faba bean (*Vicia faba* L.)	Dry-fractionated protein	59%	25%
2019	Duta et al.	Protein-enriched gluten-free pasta	Oat and faba bean (*Vicia faba* L.)	Dry-fractionated protein	42-68%	~35%
2022	Kantanen et al.	Meat analogues	Faba bean (*Vicia faba* L.)	Commercial protein isolates and dry-fractionated	55-80%	30-70%
2022	Nasrollahzadeh et al.	Meat analogues	Hemp (*Cannabis sativa* L.)	Commercial protein concentrate	61%	4:1 g:g (protein: maize starch)

2022	Nisov et al.	Meat analogues	Pea (*Pisum sativum* L.)	Commercial concentrate	49%	48%
2022	Tuccillo et al.	Meat analogues	Faba bean (*Vicia faba* L.)	Commercial protein	64%	40-70%
2021	Saldanha do Carmo et al.	Meat analogues	Faba bean (*Vicia faba* L.)	Commercial protein	64%	Water/Product feed rate ratio 3-5
2021	Ferawati et al.	Meat analogues	Faba bean (*Vicia faba* L.)	Dry fractionated proteins	56%	38-42%
2021	Zhu et al.	Meat analogues	Pea (*Pisum sativum* L.)	Dry fractionated proteins	72%	4%
2020	De Angelis et al.	Meat analogues	Pea (*Pisum sativum* L.)	Dry fractionated proteins in combination with oat protein	56%	35-70%
2022	Mefleh et al. (c)	Cheese analogues (spreadable)	Green pea (*Pisum sativum* L.)	Dry fractionated proteins combined with inulin-based emulsion-filled gel	56%	30%
2022	Mefleh et al. (a)	Dairy alternative (Fermented beverage)	Apulian black chickpea (*Cicer arietinum* L.)	Commercial dry-fractionated protein	56%	16.66%
2019	Brückner-Gühmann et al.	Dairy alternative (Yogurt-type)	Oat	Defatted dry-fractionated proteins	42%	15%
2022	Dille et al.	Gels and gelled emulsions	Faba bean (*Vicia faba* L. cv Tiffany and cv Vertigo)	Two protein concentrates combined with λ-carrageenan	65-69%	8-15%

CONVENTIONAL FOODS

Bread and bakery products

Protein fortification of bread and bakery products has been widely investigated for years, by substituting part of the wheat flour with legume flour (Boukid et al., 2019; Pasqualone et al., 2019). However, the inclusion of legume flour worsens the bread quality as a result of the dilution of gluten. Therefore, more recent studies have involved the use of protein isolates obtained by wet fractionation, which can be used in small amount to reach nutritional claims and balance the amino acid profile (Boukid et al., 2019). The utilization of dry-fractionated protein in bread and bakery products was previously investigated in different studies (Hoehnel et al., 2019; Hoehnel et al., 2020; Xing et al., 2020; Xing et al., 2021; Gangola et al., 2022; De Angelis et al., 2023). Moreover, an alternative application of the DF was studied by Ficco et al. (2018) who obtained anthocyanin-enriched flours from pigmented wheats and investigated them with respect to breadmaking, obtaining a 30–46% reduction of the glycemic index and increasing the antioxidant content of the product. Another possible way to exploit the DF in bakery products sector was proposed by Zhang et al. (2019) who concentrated the arabinoxylans of the wheat bran by electrostatic separation. Next, they substituted wheat flour at 2%, 5% and 10% with dry-fractionated arabinoxylans and used the mix in breadmaking. The wheat bread enriched with a 10% arabinoxylan fraction showed comparable quality properties to the control, and it had a two-fold higher fiber content (near 12% on a dry basis).

Hoehnel et al. (2019) carried out a comparative analysis of different plant-based protein ingredients added at 15% level of substitution of the wheat flour, also including dry-fractionated faba bean protein. The main objectives involved the characterization of the dough, as well as the evaluation of the influence during the baking process. In subsequent research, Hoehnel et al. (2020) prepared the mix of the previously studied protein (Hoehnel et al., 2019) having similar viscoelastic properties compared to the wheat flour, and they used psyllium, sugar and xylanase to produce high protein bread. The product showed enhanced nutritional value in terms of amino acid composition, antioxidant activity and low level of antinutritional compounds, as well as good sensory attributes.

Dry-fractionated chickpea proteins were applied to partially substitute wheat flour with 20–30% w/w in wheat bread (Xing et al., 2021). Interestingly, following the findings of their previous study (Xing et al., 2020), they used a sourdough produced with a solid-state fermentation with autochthonous *Pediococcus* spp., obtained by a back-slopping originating from a spontaneous fermentation. A reduction of 90% and 17% indigestible α-galactosides and phytic acid, respectively, was reported, accompanied by a 119% increase in total phenolic content (Xing et al., 2020). When this sourdough was utilized in the bread at 30% substitution of the wheat flour, the protein content of the product increased by 38.5% on a dry basis (Xing et al., 2022). However, a longer dough mixing time was needed as the substitution level increased, also causing a decrement of the specific volume as a result of a denser crumb structure. Constructively, it was acknowledged that their bread formulation cannot guarantee that the product is appealing to consumers, highlighting the need of further research to compensate the deficiencies of texture and sensory quality. With this respect, after using a mixture design, De Angelis et al. (2023) reported that a 5% inclusion of dry-fractionated pea protein in gluten-free flat bread formulation was found to be optimal for balancing the nutritional claims and the sensory quality of the product. This was also the only reference regarding the application of dry-fractionated protein in gluten-free bakery products, suggesting that further research is needed for a better exploitation of this type of ingredient for the gluten-free category.

Gangola et al. (2022) investigated the nutritional composition, and the *in vitro* starch digestibility of crackers produced with dry-fractionated faba bean protein concentrate at 40% substitution level, in comparison with the wheat-based products. Despite the improvement of the nutritional value by increasing the protein and dietary fibers contents, there was no significant difference in starch digestibility compared to the control.

Pasta

Hoehnel et al. (2020) and Gangola et al. (2021) evaluated the addition of dry-fractionated protein for the development of a nutritionally enhanced durum wheat-based pasta, whereas Duta et al. (2019) worked on the formulation of gluten-free pasta.

Hoehnel et al. (2022) substituted the 19% of semolina with a mix composed of buckwheat (13.02%) and faba bean (3.97%) proteins obtained by DF, together with lupin protein isolate (2.01%). They reported an improvement in the nutritional profile in terms of amino acid composition and protein digestibility determined by *in vivo* tests performed with rats. Overall, the use of protein mix is a good strategy for maintaining a low content of antinutritional factors. Gangola et al. (2021) investigated the effect of a 25% semolina substitution with different faba bean ingredients, including dry-fractionated protein. Apart from the obvious effects on the improvement of the protein content, an increase in the readily digestible starch and of *in vitro* hydrolytic index of starch was reported, suggesting a faster starch digestibility.

Gluten-free pasta was produced using faba bean and oat proteins obtained by DF, as well as a combination of them, with the aim of reaching the health claims, 'source of protein' and 'high protein' (Duta et al., 2019). The structural, textural, thermal and sensory properties were investigated, and it was reported that the addition of dry-fractionated oat and faba bean proteins resulted in pasta with a lower optimal cooking time (8 and 6.3 min), cooking loss (6% and 10.2% dry matter) and water absorption (152% and 147%) compared to the control prepared with oat starch. Moreover, the protein inclusion caused the hardening of the pasta, which was also chewier than the control. The glycemic index was lowered by the addition of protein ingredients, especially when the faba bean was added to the formulation. *In vitro* protein digestibility of pasta increased up to 3.5–7.1% for oat and faba bean proteins, respectively.

INNOVATIVE PLANT-BASED FOOD

Meat analogues

Plant-based meat analogues are developed to realistically imitate the quality features of meat products, in terms of texture, structure, flavor and color, even imitating the behavior of the products during cooking. The main ingredients of meat analogues are the texturized vegetable proteins (De Angelis et al., 2020; Dekkers et al., 2018) (i.e. protein with a fibrous structure mimicking the structure of the meat muscle). Texturized protein can be obtained through different structuring processes, as comprehensively described elsewhere (Dekkers et al., 2018); however, the extrusion-cooking is the most diffused. On the basis of the moisture content used in the process, it is possible to distinguish between high-moisture extrusion (i.e. using a moisture content higher than 50%) and low-moisture extrusion (i.e. 25–35% moisture content) (De Angelis et al., 2020; Dekkers et al., 2018). Most of the studies on meat analogues involve the utilization of wet-extracted proteins, with a high protein concentration. Therefore, it is not surprising that the few available studies concerning the application of dry-fractionated proteins in meat analogues are all published no earlier than 2020. For example, De Angelis et al. (2020) carried out the protein texturization by low-moisture extrusion-cooking, using a protein mix composed of dry-fractionated pea protein and oat protein in a 70:30 (w/w), comparing it with the products obtained from pea and soy protein isolates. It was reported that the process conditions were dependent on the raw materials and the use of dry-fractionated protein required a higher screw speed compared to protein isolates to obtain the fibrous texture. Moreover, the moisture contents used during the extrusion process were directly related to the water absorption capacity of the protein ingredients. Indeed, the highest moisture content was necessary to extrude the mix containing the isolates which showed the highest WAC. This reveals the importance of determining the functional properties of the ingredients for a correct set-up of the processing conditions. Overall, dry-fractionated protein led to products with a lower hardness and a more intense flavor profile compared to the extrudates obtained with the protein isolates (De Angelis et al., 2020). Finally, the importance of using low-processed and sustainable protein ingredients was highlighted, even if at a lower protein concentration compared to the protein isolates, considering that the protein content of the most diffused meat alternatives ranges from 17.7 and 25 g per 100 g.

By contrast, the high-moisture extrusion-cooking is more widely used to produce meat analogues, even starting from dry-fractionated proteins such as hemp (Nasrollahzadeh et al., 2022) and faba bean (Saldanha do Carmo et al., 2021; Saldanha do Carmo et al., 2021; Kantanen et al., 2022;

Tuccillo et al., 2022). Zhu et al. (2021) reported the production of meat analogues (i.e. plant-based protein nuggets) using a freeze structuring technique applied on an emulsion prepared with dry-fractionated pea protein in combination with other protein sources. However, this process does not appear to have been reported elsewhere.

Saldanha do Carmo et al. (2021) worked on the optimization of the high-moisture extrusion-cooking using a face-centered design varying the raw material feed rate, the moisture content and the temperature of the extruder. Based on the textural and sensory properties, the optimal temperature range was found to be 130–140 °C, with a ratio between water and the product feed rate of 4. The great influence of the moisture content on the quality of the products was also highlighted.

Nasrollahzadeh et al. (2022) found a lower anisotropy and hardness in meat analogues prepared with dry-fractionated hemp compared to the products obtained with the wet-extracted protein. These results corroborated the findings of Kantanen et al. (2022) who worked on faba bean obtained by DF and wet extraction. Nasrollahzadeh et al. (2022) also studied the water mobility of the products using low-field nuclear magnetic resonance. Interestingly, they found a higher free water content in the meat analogues prepared with the dry-fractionated protein compared to products obtained with wet-extracted protein, suggesting that this could be a favorable aspect in imitating the cooking behavior of the real meat.

Regarding the nutritional aspects, Saldanha do Carmo et al. (2020) observed a higher content of oligosaccharides in the extruded products compared to raw materials, suggesting that, during the extrusion, there was a release of oligosaccharides from other macromolecules or a modification of the food matrix. Instead, Nasrollahzadeh et al. (2022) found a higher phytic acid content when the dry-fractionated proteins were used compared to the wet-extracted protein. Therefore, the nutritional aspect is one of the drawbacks of the dry-fractionated proteins, which generally contains a higher quantity of antinutritional factors compared to the wet-extracted proteins (De Angelis et al., 2021b; Vogelsang-O'Dwyer et al., 2020; Tuccillo et al., 2022). Consequently, further research is needed to better understand the influence of the preparation process on the nutritional quality of food containing dry-fractionated protein, and technological and bio-technological approaches must be investigated to reduce the antinutritional factors in dry-fractionated ingredients.

Finally, the sensory aspects concerning the use of faba bean protein obtained by DF and wet extraction were comprehensively investigated by Tuccillo et al. (2022) In particular, they found the highest taste and aftertaste intensity, bitterness and pea flavor in the dry-fractionated protein, whereas the isolate showed off-notes, cereal flavor and odor. Moreover, the extrusion inactivated the lipid-degrading enzymes and caused the release of several volatile compounds, but did not form products of Maillard reaction, indicating that the type of ingredient was the main variable responsible for the flavor of the extrudates.

Cheese and dairy analogues

Soy-based cheese and dairy analogues such as tofu and soy milk are traditionally produced, and they are well established in the market. However, because of the concerns related to soy production and consumption, other protein sources such as pulses have been recently investigated, as comprehensively reported in recent reviews (Vogelsang-O'Dwyer et al., 2021; Mefleh et al., 2022b). Nevertheless, even in this case, there are limited studies about the utilization of dry-fractionated proteins because most of the research articles investigate the use of protein isolates.

Mefleh et al. (2022c) investigated the production of a spreadable plant-based cheese analogues formulated with dry-fractionated pea protein and a fat replacer constituted of an emulsion filled gel made with inulin and extra virgin olive oil. A higher protein content and a lower fat content were reported compared to a commercial plant-based cheese. Moreover, they analyzed some textural indices such as the spreadability index, which was similar to a dairy cheese. Finally, the impact of pea protein on sensory properties was noted, and the addition of spices such as oregano or rosemary to mask the unpleasant pea and beany flavors was proposed.

Overall, it should be reminded that imitating the melting properties of cheese is challenging for dairy analogues, considering that such properties are related to the peculiar structure given by the milk casein and by the milk fat. For this reason, cheese analogues are often firm, chalky and pasty, and these characteristics are not appreciated by consumers.

Strategies to improve both texture and sensory quality of dairy alternatives include fermentation with selected starters, as investigated by Mefleh et al. (2022a) who developed a fermented beverage using three starter cultures of lactic acid bacteria. Specifically, *Streptococcus thermophilus*, alone or in combination with *Lactococcus lactis* or *Lactobacillus plantarum*, has been used to ferment dry-fractionated chickpea protein. Fermentation led to a decrease in phytic acid content and promoted the development of exopolysaccharides able to give a higher consistency and viscosity compared to the control (i.e. sample without inoculum with starter cultures). Moreover, there was no significant decrease in the volatile compounds responsible for the beany flavor such as hexanal and 2-pentylfuran. Therefore, an optimization study concerning the flavor profile of this type of product is still needed.

Fermentation was also investigated by Brückner-Gühmann et al. (2019) who worked with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* to produce a yogurt alternative based on dry-fractionated oat protein containing 42% protein content. A protocol for structure development during fermentation was suggested, starting with a thermal-induced starch gelatinization in which the protein is incorporated. Then, during fermentation, the acidification given by the lactic acid production leads to an increase in gel strength. After the fermentation, the content of essential amino acids was unvaried and, to a limited extent, proteolysis occurred.

Conclusions

Dry-fractionated proteins show very good potential in driving the transition towards a sustainable food system. They are distinguished by distinctive techno-functional properties, including high protein solubility, excellent foaming ability, foam stability and strong gelling ability. They also absorb less water compared to the protein obtained with wet fractionation. As demonstrated by this literature review, a comprehensive understanding of these properties is crucial for effectively formulating food products and designing suitable processing technologies. From this perspective, a need to standardize the methods of analysis of the functional properties of the protein has emerged. Furthermore, DF, because of its simplicity, can also play a crucial role in valorizing minor crops and neglected species, thereby contributing to biodiversity conservation. Furthermore, this approach holds the potential to revitalize marginalized regions at the same time as aligning with agroecological and socio-economic sustainability goals. Moreover, it was interesting to note how the research has evolved, transitioning from an initial focus on the DF process to an exploration of food applications for these ingredients. However, challenges persist, particularly in the utilization of dry-fractionated proteins, especially when derived from pulses. Issues such as the presence of antinutrients and the distinctive sensory profiles, often marked by legume-related notes, remain important factors to address.

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Chapter 2. Techno-functional, rheological, and chemical properties of plant-based protein ingredients obtained with dry fractionation and wet extraction

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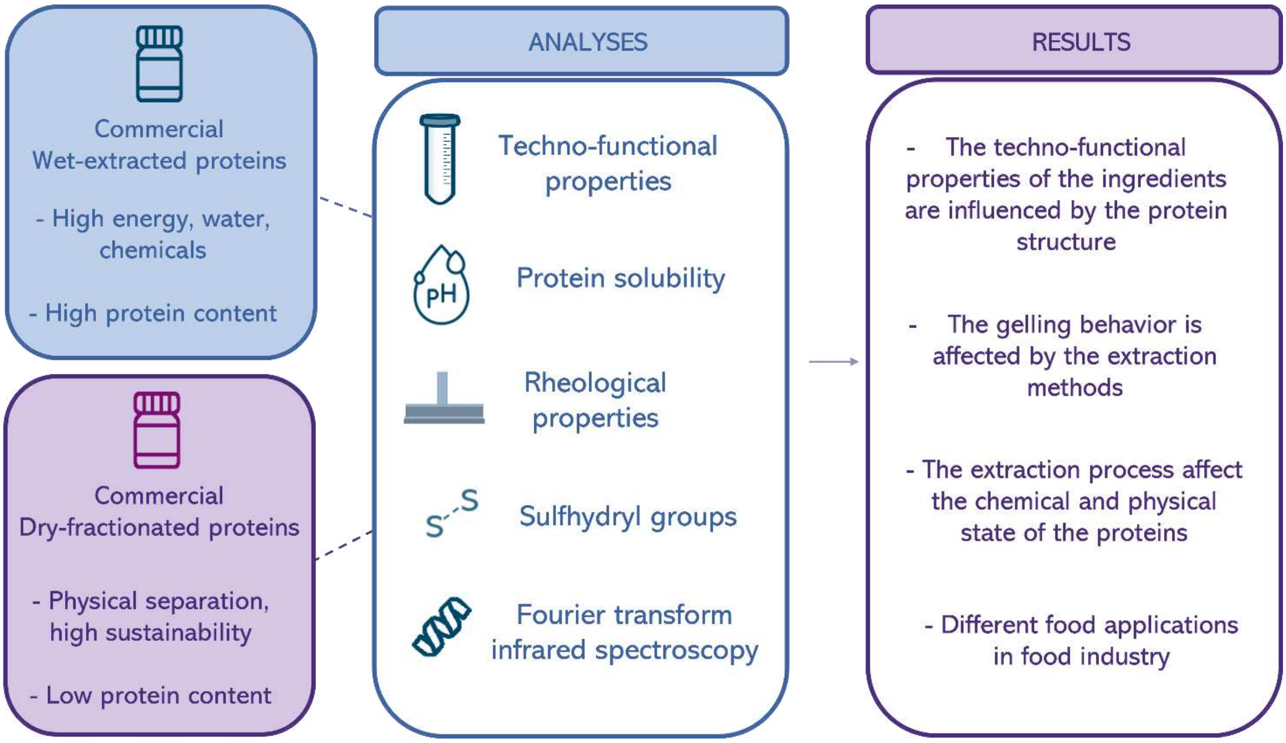
Graphical abstract



Abstract

Dry fractionation is a promising technology for producing plant protein ingredients, owing to its minimal environmental impact and adaptability to diverse plant sources. Dry-fractionated proteins are still under development with limited applications in food industry due to lack of extensive knowledge about their physicochemical, rheological and chemical properties. Wet extraction though widely used, consumes high energy, water, and chemicals. In this research, the techno-functional, rheological, and chemical properties of commercial protein ingredients of various botanical species obtained via wet extraction (WE, *n* = 8) and dry fractionation (DF, *n* = 9) were investigated in order to identify their potential food applications. Compared to DF ingredients, WE proteins showed the lowest water solubility index and protein solubility at pH 7 and 9, as well as the lowest foaming and emulsifying capacities. This behavior can be explained by the presence of denatured protein structures in WE ingredients as suggested by the analysis of the secondary structure which revealed a higher presence of random coil structures. On the contrary, the presence of non-denatured structures in combination with other constituents like carbohydrates may have contributed to the high solubility and gelling properties of the DF proteins ingredients. While wet extraction technologies can offer a wide modulation of ingredient functionality, providing a broad spectrum of food applications, dry fractionation seems to guarantee a narrow range of techno-functional properties, although with potentially higher performance in certain areas like solubility and foaming.

Keywords: Plant-based protein; dry-fractionated proteins; wet-extracted proteins; protein functionality; foaming and emulsifying properties; secondary structure.

Introduction

Plant-based protein ingredients are increasingly utilized in the food industry to address the growing demand for sustainable alternatives to animal-based products (Ma et al., 2022) and to satisfy the preferences of consumers adhering to a vegan or vegetarian diets (Ma et al., 2022). The techno-functional properties of proteins such as solubility, emulsifying and foaming capacities, water absorption, and gelling abilities, have a main role in food design and development (Ma et al., 2022; de Paiva Gouvêa et al., 2023; De Angelis et al., 2023, 2024a). Notably, these properties are dependent on the protein's chemical state and may significantly vary depending on the extraction methods employed (Ma et al., 2022) and the plant species (do Carmo et al., 2020). As recently reported plant-based protein ingredients are obtained in the form of protein concentrates or isolates (Ma et al., 2022), which are mainly produced via wet extraction technologies (Yang et al., 2024). Specifically, the alkaline extraction followed by the isoelectric precipitation is one of the most diffused wet extraction protocols. These processes are carefully described elsewhere (Boye et al., 2010b; Ma et al., 2022; Yang et al., 2024). Briefly, the raw material is dispersed in alkalinized water to maximize protein solubilization. Then, after the separation of the insoluble components, the proteins are precipitated lowering the pH to the isoelectric point, and subsequently neutralized. It appears evident that the production process is complex and has a non-negligible environmental footprint (Vogelsang-O'Dwyer et al., 2020), which contrasts with the purposes of increasing the sustainability of the agri-food system. For this reason, there are emerging studies that propose and evaluate alternative extraction technologies (De Angelis et al., 2024a), with dry fractionation resulting as the most sustainable one (Lie-Piang et al., 2021). Dry fractionation is based on a physical separation of a finely milled flour where the protein bodies are detached from starch granules, fibers and other non-protein components. Although this is indeed the aim, fully separating protein bodies and other constituents like carbohydrates, fibers, and lipids, is not possible in practice, leading to the formation of fractions with a complex composition. The separation can be achieved based on density and particle size in the air classification (Schutyser et al., 2015), or by exploiting the charging properties of components in tribo-electric separation (Tabtabaei et al., 2019).

Being based on two different working principles, dry fractionation and wet extraction technologies generate ingredients with different chemical properties and functionality. Specifically, the pH shifts and high temperatures used in wet extraction can lead to protein denaturation, causing the unfolding of the native protein structure and the exposure of hydrophobic regions, sulfhydryl groups, and the formation of aggregates which significantly affect the techno-functional properties of the protein (Ma et al., 2022; Vogelsang-O'Dwyer et al., 2020; De Angelis et al., 2024a; Yang et al., 2024). By contrast, dry fractionation preserves the native structure of the proteins. Moreover, the lower protein content compared to wet-extracted proteins and the presence of other components such as starch, fibers, and lipids, also contribute to modulating their behavior in various food applications (De Angelis et al., 2023; Ma et al., 2022).

Although the techno-functional properties of plant-based protein concentrates/isolates obtained through wet extraction and dry fractionation have been studied by various authors (Ladjal-Ettoumi et al., 2016; Tang et al., 2021; Chang et al., 2022; Silventoinen et al., 2021), it is essential to note that the raw materials used in these investigations were obtained through lab-scale extraction processes, not from commercial ingredients. In fact, the extraction yields, and quality of the ingredients can be largely different between an industrial scale and a laboratory scale. Recently, Xu et al. (2022) and Jakobson et al. (2023) have compared techno-functional and rheological properties between commercial isolates and concentrates. Xu et al. (2022) compared the physicochemical, structural, and techno-functional properties of hemp protein to those of commercial plant and animal proteins available in the U.S. market. Meanwhile, Jakobson et al. (2023) focused on the sensory and techno-functional characteristics of commercial plant protein ingredients mainly obtained with wet extraction technology, with only three samples obtained using dry fractionation. Among these ingredients, the authors studied different batches of wet extracted oat and pea proteins, finding significant differences in functionality of the latter, possibly related to the stability of the production process. Overall, the information on commercial dry-fractionated protein ingredients is still limited in the literature. Additional research is needed to better understand the relationship between their techno-functional and chemical properties, and to explore ways to promote their broader adoption in the food industry.

Therefore, the aim is to investigate the techno-functional, rheological and chemical properties of a collection of 17 commercial protein ingredients produced through both dry fractionation and wet extraction. The characterization has two specific objectives that include i) the exploration of the structure-related and compositional factors that may explain the functional behavior of these ingredients, and ii) the identification of potential applications as ingredients in various food products based on these techno-functional properties.

Materials and methods

Protein collection and chemical determinations

Commercial protein concentrates were purchased online from different retailers operating in the European market, especially in Italy and Spain. According to the information provided by the suppliers, the protein ingredients were categorized into two groups: proteins produced through dry fractionation, involving milling and air classification (DF, *n*=9), and proteins obtained via wet extraction n (WE, *n*=8). In general, these samples were representative of the most commonly used protein ingredients in the food industry for various food applications. The list of the protein ingredients, along with their protein and lipid content is reported in Table 1.

Techno-functional properties

Water absorption index (WAI), water solubility index (WSI), and oil absorption capacity (OAC) of the protein ingredients were performed as described in Summo et al. (2019). In order to determine WAI and WSI 1.75 g of ingredient was mixed and 15 mL of distilled water in pre-weighed centrifuge tubes. The mixture was heated at 70 °C for 30 min and then centrifuged at 3000×*g* for 20 min. WAI was calculated as the ratio of the weight of the sediment to the weight of the protein ingredient. Meanwhile, the supernatant was transferred into a pre-weighed evaporating dish and dried overnight at 105 °C. WSI is expressed as percentage ratio of solid content of the supernatant relative to the weight of the ingredient.

To determine OAC, 0.75g of protein ingredients were suspended with 9 mL of peanut oil in pre-weighed centrifuge tubes. The tubes were stirred for 1 min and again after 30 min, and finally centrifuged at 3000×*g* for 20 min. The excess oil was carefully drained, and the pellet was weighed. OAC was expressed as gram of oil bound per gram of protein ingredient.

Protein content (N × 6.25) of the protein ingredients was determined according to the method AOAC 979.09 (AOAC, 2006). The lipid content was determined by a Randall apparatus (SER 148 extraction system, Velp Scientifica srl, Usmate, Italy) according to the method AOAC 945.38F (AOAC, 2006), using diethyl ether as extracting solvent. Ash content was determined according to the AOAC method 923.03 (AOAC, 2006) and carbohydrate content was determined by difference, subtracting the content of protein, lipid and ash to 100.

Water absorption capacity (WAC) was performed following the official method AACC method no. 51–61 (AACC, 1990). Briefly, 5 g of ingredients were suspended and 25 mL of distilled water in pre-weighed centrifuge tubes. The mixture was stirred for 5 min and then centrifuged at 1000×*g* for 15 min. The supernatant was discarded, and the sediment weighed. WAC was expressed as a gram of water bound per gram of protein ingredient.

Table 1. List of the plant proteins obtained with wet extraction and dry fractionation, protein and lipid contents.

	Extraction Method	Protein Content (g/100g d.m.)	Lipid content (g/100g d.m.)	Ash (g/100g d.m.)	Carbohydrates (g/100g d.m.)
Oat	Wet extraction (WE)	54.34	16.20	4.58	24.88
Chickpea (Supplier 1)		90.42	0.90	0.47	8.21
Chickpea (Supplier 2)		70.87	10.26	2.35	16.52
Lentil		82.40	0.40	3.26	13.94
Pea		86.09	0.31	9.18	4.42
Hemp		56.59	10.77	8.55	24.09
Soy		84.36	0.40	5.37	9.87
Wheat gluten		74.57	1.28	0.81	23.34
Chickpea	Dry fractionation (DF)	56.84	7.61	5.15	30.40
Lentil (55%)		55.60	2.33	3.77	38.30
Lentil (60%)		62.60	2.60	5.20	29.60
Pea (55%)		55.88	2.59	2.81	38.72
Pea (60%)		60.41	3.01	3.74	32.84
Mung bean		56.90	2.23	2.85	38.02
Grasspea		55.30	1.35	3.01	40.34
Faba bean (55%)		55.22	2.30	2.91	39.57
Faba bean (65%)		65.80	2.67	5.60	25.93

Foaming ability (FA) and foam stability (FS) at 10 (FS10) and 20 (FS20) min were measured as described in De Angelis et al. (2022) with slight modifications. A dispersion of sample (5% w/v) was prepared into a beaker and whipped with an Ultra-turrax (T-25, IKAWerke GmbH & Co. KG, Staufen, Germany) at 24,000 rpm for 90 s. Immediately, the whipped sample was poured into a 50 mL graduated cylinder. The glass container was washed with 5 mL of distilled water to recover the remaining foam and poured into the graduated cylinder. FS10 and FS20 are calculated as the percentage ratios of the foam volume at 10 and 20 min, respectively, to the initial foam volume. FS10 and FS20 as the percent ratio between the foam volume at 10 and 20 min and the initial foam volume.

Emulsion capacity (EC) was evaluated as described in Alfaro-Diaz et al. (2021) with some modifications. A 1% protein dispersion was prepared in a 50 mL centrifuge tube with a homogenizer (T-25, IKAWerke GmbH & Co. KG, Staufen, Germany) at 24,000 rpm for 3 min. Then, an amount of oil is added to the solution, and homogenize for 3 min at 24,000 rpm. Immediately, after homogenization the conductivity was measured with a HI2003 conductivity meter equipped with a probe with four ring potentiometric measuring system (HI 763100, Hanna Instruments, Villafranca Padovana, Italy). The addition of the oil continued until the inversion point, i.e., the switch from an oil-in-water emulsion to a water-in-oil emulsion, which results in a drop in conductivity. EC is measured as:

$$EC = \frac{g\ of\ added\ oil}{g\ of\ sample}$$

Protein solubility

Protein solubility was determined at different pH values following the method described in Tas et al. (2022) with some modifications. Briefly a 5% (w/v) of sample aqueous dispersion (based on the protein content of the ingredient) was prepared and adjusted at four different pH i.e., 3, 5, 7, 9 by using 0.1 N NaOH or HCl. The dispersion was stirred for 10 min, then centrifugated at 18,000×*g* for 5 min and the supernatant was collected. A 100 µl of supernatant was mixed with 900 µl of water and 5 ml of Lowry reagent (Merck KGaA, Darmstadt, Germany) and incubated for 20 min at 25°C in dark conditions. Then 250 µl of Folin-Ciocâlteu reagent (Merck KGaA, Darmstadt, Germany) was added and incubated for 30 min at 25°C in dark conditions. The samples were centrifuged at 15,000×*g* for 5 min and the absorbance was measured at 750 nm by using a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The value was calculated by using a calibration curve (R^2 =0.997) with Bovine Serum Albumin (Merck KGaA, Darmstadt, Germany) as standard. Protein solubility was calculated as the percent ratio of soluble protein in the supernatant as respect to the total protein content in the sample.

Rheological evaluation of the gelling behavior

Temperature sweep analysis was carried out following the method described in Schlangen et al. (2022) with some modifications. A 15% (w/v) aqueous dispersion of the sample was prepared using an Ultra-Turrax (T-25, IKAWerke GmbH & Co. KG, Staufen, Germany) at 24,000 rpm for 30 min. The dispersion was refrigerated at 4 °C overnight to perfectly hydrate the powders and were briefly agitated with a vortex before the analysis. The temperature sweep was conducted using a HAAKE MARS iQ Air rheometer (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) equipped with coaxial cylinders geometry (CC25 DIN/Ti) with 5.3 mm gap between the two geometries and using a strain value of 0.5%, within the linear viscoelastic region of the materials. The dispersion was heated from 25 °C to 95 °C at a heating rate of 3 °C/min. Then, the sample was held at 95 °C for 6 min, followed by a cooling step from 95 °C to 25 °C at a rate of 3 °C/min. To observe the gelation, the crossover points between G’ and G’’ (Pa) was considered. The analysis was carried out in duplicate, preparing two distinct dispersions of each ingredient.

Total, free sulfhydryl group (SH) and disulfide bond (SS) contents

The determination of total, free -SH, and SS content was conducted following the method described by Gao et al. (2020) with some modifications. 30 mg of protein ingredients were solubilized in 10 mL of Tris-Gly buffer (0.086 M Tris, 0.09 M glycine, 0.004 M Ethylenediaminetetraacetic acid (EDTA), pH 8.0) containing 8 M urea and gently stirred for 2 h. For measuring free -SH content, 1 mL of the protein solution was mixed with 150 µL of Ellman's reagent (2 mM 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and 50 mM sodium acetate dissolved in water) and incubated for 20 min at 25 °C in the dark. Then, the samples were centrifuged at 15,000×*g* for 10 min, and the absorbance was measured at 412 nm using a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). To determine the total -SH content, 1 mL of the protein solution was added to 4 mL of Tris-Gly buffer and 50 µL of 2-mercaptoethanol and incubated for 1 h at 25 °C in the dark. The mixture was added to 10 mL of 12% trichloroacetic acid (TCA) solution, incubated for 1 h, and then centrifugated at 15,000×*g* for 5 min. The resulting precipitate was washed twice with 5 mL of TCA solution and after that, the precipitate was dissolved in 10 mL of Tris-Gly buffer. 150 µL of Ellman's reagent were added to 4 mL of the protein solution obtained and then incubated for 20 min in a dark room. The samples were centrifuged at 15000×*g* for 10 min and the absorbance was measured at 412 nm. The content of total and free -SH was quantified using a calibration curve (R^2 =0.998) prepared with standard concentrations of L-cysteine hydrochloride monohydrate (Merck KGaA, Darmstadt, Germany). The disulfide bond (SS) content was calculated by subtracting the free -SH content from the total -SH content and dividing the result by two (Gao et al., 2020).

Fourier transform infrared spectroscopy (FTIR)

The secondary structure of the dry ingredient powders was characterized using the Attenuated Total Reflectance moduli on a FTIR spectrophotometer (Nicolet iS50, Thermo Fisher Scientific, Waltham, USA), supported by OMNIC software. The acquisition conditions were: 1000-2000 cm^{-1} spectral range, 4 cm^{-1} resolution, 32 scans per samples. Four spectra per each sample were collected at room temperature, recording a new background (32 scans) after every sample. The data elaboration was carried out using The Unscrambler X (v. 10.2, AspenTech, Bedford, USA) considering the amide I region (1600-1700 cm^{-1}). The mean spectra of four replicates were subjected to an area normalization to remove any possible effect of protein concentration (Cai & Singh, 2004). Then, the second order derivative was applied with the Savitzky-Golay algorithm (second order polynomial and 3 smoothing points) to obtain the information related to the secondary structure. Exploratory analysis using principal component analysis (PCA) was carried out to describe qualitative differences between DF and WE proteins.

Statistical analysis

All the data are expressed as a mean of three technical replicates having a standard deviation < 5%. The means of all DF and WE proteins were processed using one-way analysis of variance ANOVA followed by Tukey's HSD (Honestly Significant Differences) test for multiple comparisons at a significance level α = 0.05 by using Minitab 19 Statistical Software (Minitab Inc., State College, PA, USA). Pearson's correlation of all data was carried out at 95% confidence interval using the same software. Violin plots of the data were generated using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA) and the same software was used to compute the PCA of the FTIR spectral data.

Results and discussion

Techno-functional properties of protein ingredients

Figure 1 depicts the violin plots of the techno-functional properties of the two classes of commercial plant-based protein ingredients, whereas Table 2 reports the mean value of each sample together with the results of the statistical analysis between the two groups. The water absorption index (WAI) showed no significant differences between WE and DF proteins. A large variability was observed in the WE samples (Figure 1), which ranged from 2.55 g/g in chickpea protein to 7.50 g/g in soy protein. By contrast, a lower variability was observed in the DF samples, where the WAI ranged from 2.09 g/g in chickpea (55%) to 3.51 g/g in pea (60%). WAI is commonly associated with the ability of the molecules to retain water after the heating process in excess of water, thereby explaining the gelling capacity of the material (Du et al., 2014). Further discussion about this aspect is given in the section 3.2.

Water solubility index (WSI) indicates the quantity of solids remaining soluble after the heating process and it is inversely correlated to WAI (Summo et al., 2019). In fact, the WE proteins exhibited the lowest WSI (Table 2). Two possible factors may contribute to these results. First, the protein, which are the primary component in the ingredients studied, may have undergone denaturation caused by changes in the pH values and high temperature during the extraction. This denaturation likely exposed hydrophobic groups, reducing their solubility (Nasrollahzadeh et al., 2022). On the contrary DF proteins are characterized by a preserved native structure (Vogelsang-O'Dwyer et al., 2020; De Angelis et al., 2023), which explain the highest WSI and lowest WAI values. Secondly, the higher WSI value in the DF ingredients may be attributed to the presence of soluble non-protein constituents in the fractions, like carbohydrates and minerals (Solaesa et al., 2020). In fact, a positive correlation was observed between the WSI value and the carbohydrates content ($r = 0.548$; $p = 0.023$). It is worth noting that the extraction process can predominantly influence the protein functionality, even considering the same species (not to mention the intraspecific quali-quantitative variation of protein). In fact, as an example, WE chickpea (Supplier 1) had a WSI of 32.18% while WE chickpea (Supplier 2) had a WSI of 7.15%. It is even reported that different batches from the same supplier may affect the protein functionality (Jakobson et al., 2023), pointing out the importance of monitoring the techno-functional properties of the raw materials during the quality control of industrial processing.

Owing to the same reason, a similar behavior was observed when the analysis was conducted at room temperature (water absorption capacity – WAC). WAC was significantly higher in the WE proteins (2.98 g water/g) compared to DF proteins (0.77 g water/g) highlighting important differences in the ingredient interaction with water. Again, a large variability among the WE proteins was found (Figure 1), ranging between 1.46 g/g in wheat gluten and 4.71 g/g in pea, whereas in the DF proteins WAC ranged from 0.42 g/g in faba bean (65%) to 0.98 g/g in grasspea. The overall results are in line with previous studies of dry-fractionated pea and faba bean (do Carmo et al., 2020) and other pulse proteins (De Angelis et al., 2021) and hemp proteins obtained with dry and wet extraction (Nasrollahzadeh et al., 2022).

The information about water absorption supports the optimization the food processing conditions and the formulation of food. For instance, processes in which a certain amount of water is mixed with the ingredients, such as the extrusion-cooking (De Angelis et al., 2023; De Angelis et al., 2024d) requires the knowledge of the water absorption. Moreover, a high WAC is reported to be desired in meat analogues because it prevents cooking loss and shrinkage, as well as in bakery products (Ma et al., 2022) but could also be considered a drawback in foods in which the proteins need to remain soluble, rather than absorbing water, such as vegetable beverages.

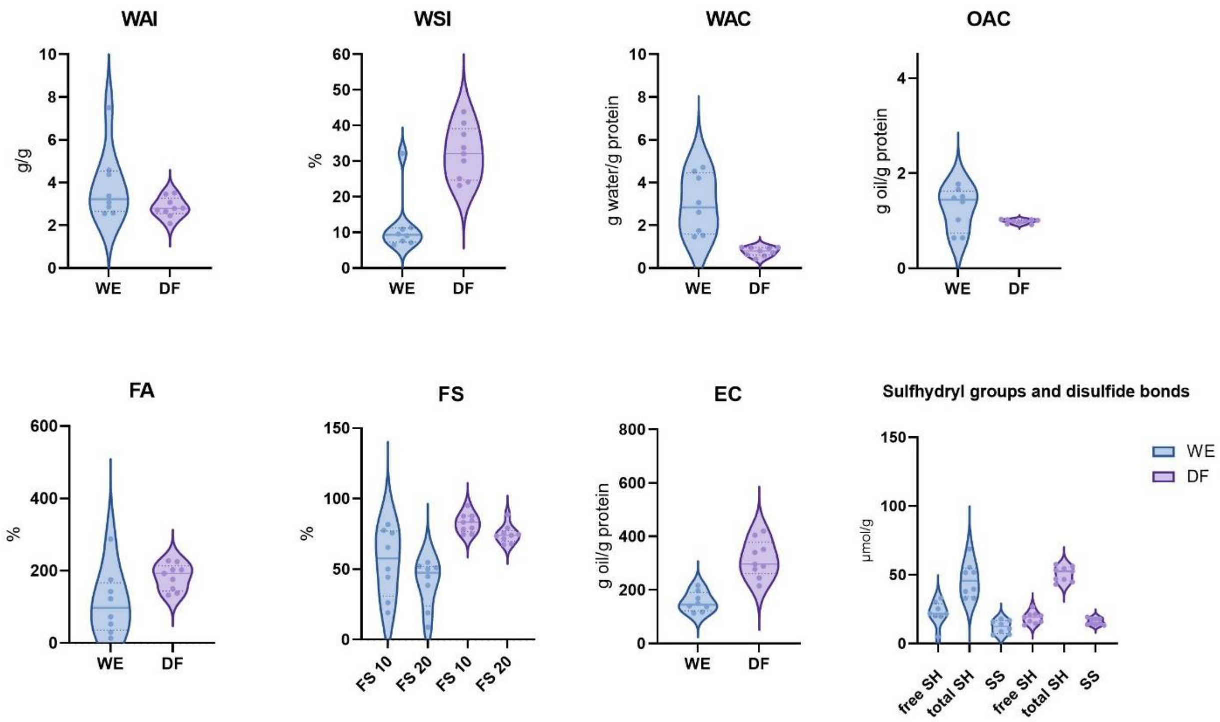


Figure 1. Violin plot illustrating the distribution of the techno-functional properties of wet-extracted (WE) and dry-fractionated (DF) ingredients. WAI: water absorption capacity; WSI: water solubility index; OAC: oil absorption capacity; FA: foaming ability; FS: foaming stability; EC: emulsion capacity, SH: sulfhydryl groups; SS: disulfide bonds. The width of the 'violin' represents the data density, providing insights into the probability distribution of the variable within each category.

Oil absorption capacity (OAC) indicates the capacity of the ingredient to hold oil. No significant differences were observed between the WE (1.27 g oil/g) and DF proteins (0.99 g oil/g). However, a large distribution of WE samples is observed (Figure 1), suggesting again that an advantage of wet extraction technologies is the possibility to modulate the functionality of the ingredients. The overall results are in line with what was previously observed by do Carmo et al., (2020) and Tabtabaei et al. (2019) for DF proteins and Paiva Gouvêa et al., (2023) and Alfaro-Diaz et al., (2021) for wet extracted proteins. Interestingly Xu et al., (2022) studied the OAC of whey and albumin protein isolates, finding similar values compared to the plant-based ingredients used in this study, suggesting possible application in plant-based mayonnaise or dairy analogs (De Angelis et al., 2024a) in which an OAC similar to the animal proteins is required.

Protein solubility is presented in Figure 2, while the mean values and the result of statistical analysis at the different pH are reported in Table 2. In general, DF proteins showed the highest solubility at pH 7 and 9. This trend corroborates what was previously observed on dry and wet extracted proteins and flours, e.g., faba beans (do Carmo et al., 2020), lentil, chickpea, and pea isolates and flours (Boye et al., 2010a). At pH 5 both the ingredient types showed low and similar solubility because the pH conditions were near the isoelectric point of the proteins (Ma et al., 2022). An exception is represented by the wheat gluten, that showed the lowest solubility at pH 7, because the isoelectric point of this protein is reported to be at pH 6-7 (Hardt et al., 2013). We give different hypotheses to explain these results. Firstly, DF proteins usually have a higher content of highly-soluble albumin fraction compared to WE ones, because this fraction is generally separated during wet extraction procedures, whereas with dry fractionation is not (Boye et al., 2010a; do Carmo et al., 2020; Yang et al. 2021). Secondly, the hydrophobic regions of the protein tend to expose after wet extraction, causing a reduction of the protein solubility (Vogelsang-O'Dwyer et al., 2020). Thirdly, Yang et al.

(2024) reported that protein aggregates can be formed via wet extraction processes, and they can reduce the protein solubility. Information about the protein solubility are useful for the development of beverages such as protein-based beverages or milk substitutes, in which the solubilization of the material is required (Ma et al., 2022). Specifically, if the beverage has a neutral pH, the use of DF ingredients might be preferable, whereas for fermented or acidic beverages, we could hypothesize a similar behavior between DF and WE ingredients.

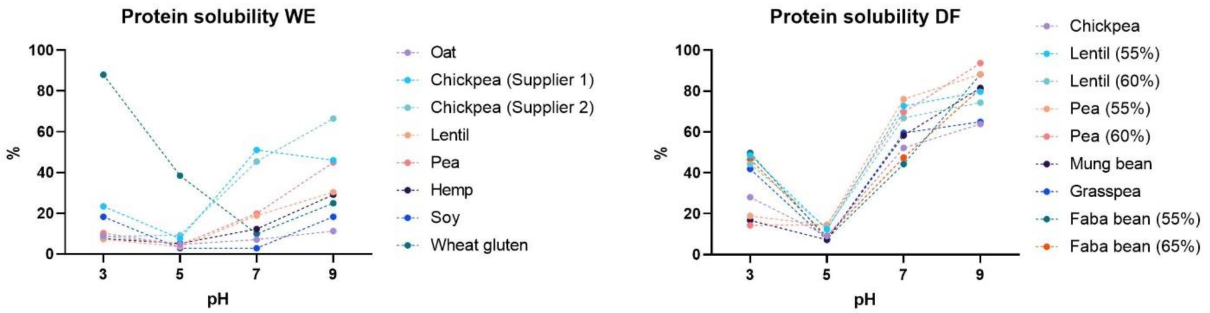


Figure 2. Protein solubility (expressed as percentage) of wet-extracted (WE) and dry-fractionated (DF) ingredients determined at different pH conditions.

Foaming ability (FA) and foaming stability (FS) are reported in Table 2. DF ingredients had a significantly higher FA (182.92 %) than WE ingredients (112.03 %), and, importantly, longer stability of the foam, as highlighted by the values of FS after 10 and 20 minutes. The good foaming properties of DF ingredients suggest a higher availability of albumin-type proteins, which are better preserved during the dry fractionation process compared to the WE technology (Vogelsang-O'Dwyer et al., 2020; Yang et al., 2021; Yang et al., 2023). In fact, Yang et al. (2021) and Yang et al. (2023) suggested that during the alkaline extraction followed by the isoelectric precipitation, the albumin fraction is separated from the globulin fraction.

The high foam stability of all the DF ingredients indicates a good capacity of the proteins to create a cohesive film that delays the coalescence of the air bubbles (Silventoinen et al., 2018; de Paiva Gouvêa et al., 2023). This stability may be further supported by the presence of carbohydrates, which enhance the viscosity of continuous phase between air bubbles (Silventoinen et al., 2018; de Paiva Gouvêa et al., 2023). Figure 1 shows a wide variability in the FA of WE samples, ranging from 13.75% in hemp to 287.50% in wheat gluten. Kolpakova et al., (2016) reported a FA value of 283% for the egg albumin, whereas de Paiva Gouvêa et al., (2023) reported a range of ~75-90% FA in soy protein isolate and concentrate. A similar FA of wheat gluten was found by Kolpakova et al. (2016), that reported a value of 174-200%. The lipid content of the ingredients (Table 1) and FA were negatively correlated (r = -0.634; p = 0.006). In fact, chickpea (Supplier 2), hemp and oat had a high lipid content (10.8, 10.8, 16% respectively) and low foaming ability (30.00, 13.75, 52.50% respectively). Shevkani et al., (2014) explained that lipids are more active surface than proteins and, consequently, they could be faster adsorbed at interface, hampering the adsorption of proteins during foaming. Overall, the high foam ability and stability of DF protein ingredients can be exploited to substitute whey and egg proteins that are usually used as whipping agents in baked goods, mousses, or other foods that require the incorporation of air in the food matrix (Ma et al., 2022; de Paiva Gouvêa et al., 2023; De Angelis et al., 2024b).

Table 2. Techno-functional properties of dry-fractionated (DF) and wet-extracted (WE) proteins.

		WAI (g/g)	WSI (%)	WAC (g water/g)	OAC (g oil/g)	Protein solubility at pH 3 (%)	Protein solubility at pH 5 (%)	Protein solubility at pH 7 (%)	Protein solubility at pH 9 (%)	FA (%)	FS10 (%)	FS20 (%)	EC (g/g)
WE	Oat	3.37	11.38	1.53	1.02	8.90	4.67	7.13	11.27	52.50	19.08	19.08	113.37
	Chickpea (Supplier 1)	2.55	32.18	1.75	1.51	23.35	7.63	51.03	46.03	142.50	26.30	8.79	149.44
	Chickpea (Supplier 2)	3.07	7.15	3.06	0.64	8.63	9.25	45.39	66.49	30.00	50.00	50.00	136.93
	Lentil	4.38	6.50	4.21	1.77	7.28	3.63	18.84	30.34	175.00	44.29	38.57	216.86
	Pea	4.59	9.05	4.71	1.40	10.27	4.56	19.86	44.96	72.50	65.49	44.78	197.72
	Hemp	2.58	10.91	2.61	1.48	7.52	5.19	12.28	29.11	13.75	81.72	54.85	117.42
	Soy	7.50	9.60	4.53	1.66	18.32	2.91	2.85	18.31	122.50	77.52	51.01	168.86
	Wheat gluten	2.86	7.62	1.46	0.64	87.95	38.49	9.96	24.94	287.50	75.65	52.18	140.08
DF	Chickpea	2.09	43.89	0.63	0.99	28.07	9.03	52.23	63.83	176.25	95.04	88.66	296.32
	Lentil (55%)	2.79	30.15	0.92	1.01	48.52	12.21	72.72	79.75	137.50	74.54	67.27	288.91
	Lentil (60%)	2.71	33.76	0.80	1.02	44.26	12.94	66.85	74.41	225.00	83.33	75.56	340.28
	Pea (55%)	3.46	23.18	0.95	0.99	18.85	14.23	76.02	88.29	150.00	78.33	71.67	244.91
	Pea (60%)	3.51	24.20	0.97	0.97	14.33	14.53	69.64	93.74	192.50	84.44	79.22	277.64
	Mung bean	2.65	32.11	0.57	1.03	16.71	7.20	58.29	81.57	227.50	74.72	74.72	404.90
	Grasspea	2.45	25.15	0.98	1.02	41.85	7.17	59.54	64.98	202.50	87.65	74.08	215.63
	Faba bean (55%)	3.08	37.50	0.70	0.93	49.82	7.82	44.27	88.22	132.50	79.22	73.58	351.64
	Faba bean (65%)	2.80	40.71	0.42	0.92	46.59	8.93	47.43	81.09	202.50	87.67	67.92	420.46
	WF	3.86 ± 1.66a	11.80 ± 8.42b	2.98 ± 1.36a	1.27 ± 0.44a	21.53 ± 27.46a	9.54 ± 11.88a	20.92 ± 17.82b	33.93 ± 17.74b	112.03 ± 90.46b	55 ± 23.94b	39.91 ± 17.01b	155.08 ± 36.97b
	DF	2.84 ± 0.46a	32.29 ± 7.41a	0.77 ± 0.20b	0.99 ± 0.04a	34.33 ± 27.03a	10.45 ± 11.72a	60.78 ± 19.85a	79.54 ± 17.88a	182.92 ± 35.97a	82.77 ± 6.77a	74.74 ± 6.40a	315.63 ± 69.28a

Data are expressed as a mean (*n*=3) with a standard deviation <5% of the three technical replicates. Different letters for the same parameter mean significant differences according to the post-hoc Tuckey's HSD test at $p < 0.05$. WAI= water absorption capacity; WSI= water solubility index; OAC=oil absorption capacity; FA=foaming ability; FS=foaming stability at 10 min (FS10) and 20 min (FS20); EC=emulsion capacity.

Emulsion capacity (EC) is also presented in Table 2. Higher values of EC were observed in the DF ingredients (315.63 g oil/g) compared to the WE ones (155.08 g oil/g). The highest value (420.46 g/g) was observed in faba bean (65%) and the lowest value (215.63 g/g) in grasspea. For what concerns the WE protein ingredients, the highest value was observed in lentil (216.86 g/g), whereas oat protein showed the lowest EC (113.37 g oil/g). It was previously reported that EC of the egg yolk is 107.1 g/g (Fu et al., 2020). As in previous findings, the physicochemical state of the proteins as well as the chemical composition of the ingredients likely explain these results. Specifically, the main contribution to the EC comes from the behavior of proteins in emulsions where they create a viscoelastic layer at the oil/water interface, preventing coalescence. A high protein solubility could enhance this effect (Geerts et al., 2017). To support this hypothesis, a positive and significant correlation was found between EC and protein solubility at pH 7 (r = 0.597; p = 0.011), and pH 9 (r = 0.759; p < 0.001). On the other hand, denatured protein tends to form aggregates that reduce the interfacial properties, leading to the formation of inhomogeneous layer and as results cause a minor stabilization of the emulsion that is subject to flocculation, and coalescence (Geerts et al., 2017). Interestingly, the residual non-protein components present in the DF ingredients could additionally promote the EC, serving as stabilizing thickening agents of the system (Funke et al. 2022). In our case, a positive correlation was found between the carbohydrate content and EC (r = 0.548; p = 0.023). We might also speculate that the composition the DF materials may contribute to emulsion stabilization through the presence of solid microparticles, which can play a stabilizing role (Dickinson 2013). Consequently, DF proteins are convenient for emulsified foods such as sauces, salad dressings (De Angelis et al., 2022), and mayonnaise (Ma et al., 2022).

Total, free sulfhydryl group (SH) and disulfide bond (SS) contents

The total, free SH and SS bonds of the protein samples are presented in the Table 4. In general, the comparison between the means of WE and DF protein ingredients displayed significant differences only for the SS bond content, and it can be observed a high data variability among the WE samples (Figure 1). In fact, the free SH content ranged from 4.88 µmol/g for oat protein to 33.05 µmol/g for hemp protein. Total SH content ranged from 32.81 µmol/g for chickpea protein to 68.81 µmol/g for hemp protein. In DF proteins the free SH content ranged from 13.41 µmol/g for faba bean (55%) to 26.80 µmol/g for pea (60%), whereas the total SH content varied from 43.09 µmol/g for faba bean (55%) to 57.52 µmol/g for mung bean. The literature reports similar values for pea, chickpea and lentil protein isolates (Ladjal-Ettoumi et al., 2016), hemp and soy isolates (Xu et al., 2022) and pea protein fractions (Kornet et al., 2021). The content of sulfhydryl groups can vary among samples due to several factors including the amino acid composition (i.e., the presence of sulfur amino acids) and the genotype considered (Ladjal-Ettoumi et al., 2016; Cui et al., 2020; Xu et al., 2022). Moreover, for the purposes of this research, the assessment of the sulfhydryl groups could support the hypothesis discussed in the next paragraphs concerning the conformational structure of the protein (Gao et al., 2020; Chang et al., 2022). Sulfhydryl groups are influenced by the extraction procedures (Jiang et al., 2017; Cui et al., 2020; Gao et al., 2020) and they are involved in the reactions caused by protein unfolding during heat-induced gelation process, while disulfide bonds contribute to the formation of strong gels (Yang et al., 2021). It has been previously reported that high-alkaline conditions during extraction (e.g., alkaline extraction and isoelectric precipitation process) can result in the cleavage of disulfide bonds and in an increase of free SH, that can be considered an indicator of conformational changes in the protein structure (Gao et al., 2020). Therefore, a higher free sulfhydryl group content may be related to the exposure of internal groups due to protein unfolding together with the breakdown of disulfide bonds (Cui et al., 2020; Gao et al., 2020). This trend was not clearly visible in our samples, also considering that the differences detected were not significant, because of the high variability within the class of proteins. For instance, if the oat protein were excluded from the data analysis – because of its very low content of free SH groups compared to the other proteins – the differences in free SH would become significant (data not shown). This might confirm the hypothesis that WE proteins were subjected to denaturation during extraction (possibly confirmed by the high free SH content), corroborating what was previously discussed with the techno-functional properties and the rheological behavior.

Rheological evaluation of the gelling behavior

The gelation behavior of the ingredient dispersions subjected to a temperature ramp is depicted in Figure 3. DF ingredients showed uniform behavior during the temperature sweep analysis, whereas more heterogeneous behavior was found in the WE protein ingredients. In particular, DF proteins displayed heating/cooling patterns characterized by an increase in storage modulus (G′) and loss modulus (G′′) at around 60-70 °C. In this temperature range, the crossover point is observed, where G′ becomes higher than G′′, indicating the transition from a viscous material to a semi-solid elastic gel (Schlangen et al., 2022; Shrestha et al., 2023). The protein gelation phenomena have been carefully described in previous works concerning both dry-fractionated (Schlangen et al., 2022) and wet extracted proteins (Yang et al., 2021), finding similar heating-related behavior. Briefly the initial increase of the G′ and G″ can be attributed to the starch gelatinization (Schlangen et al., 2022). As the temperature increases to 78-83 °C the protein denaturation begins, causing the loss of the globular structure due to its unfolding (Schlangen et al., 2022). Specifically, during the heating and denaturing process, the protein structure exposes functional groups like sulfhydryl and cysteine residues and hydrophobic regions (Chang et al., 2022; Schlangen et al., 2022; Yang et al., 2024). Consequently, the development of both covalent bonds, such as disulfide bonds (O'Kane et al., 2005), and non-covalent interactions like hydrophobic interactions, hydrogen bonds, and van der Waals forces are observed (Schlangen et al., 2022; Yang et al., 2021). These interactions are responsible for the gel formation (detected by the increase of G′). Both G′ and G′′ continue to increase during the cooling step due to interactions involving the unfolded protein molecules in addition to the cold-induced strengthening of the gel structure (Shrestha et al., 2023). Since the protein unfolding is a crucial step for protein gelation (Yang et al., 2021), denatured proteins have lower gelling abilities compared to native ones. The presence of carbohydrates and non-protein components in DF ingredients may also contribute to the overall gel structure and properties (Yang et al., 2021; Schlangen et al., 2022). While carbohydrates can impede the protein-protein interaction in protein gels (Yang et al., 2021), starch may enhance gel network. Schlangen et al. (2022) previously reported that gels formed from DF materials with high starch content exhibited a longer linear viscoelastic regime compared to those with higher protein content, suggesting that the network formed was more resistant to deformation. Among wet extraction procedures, alkaline extraction and isoelectric precipitation may result in ingredients with lower gelling ability compared to salt extraction followed by micellar precipitation or ultrafiltration (Yang et al., 2021). These findings explain the heterogenous behavior observed in WE proteins (Figure 3), even within samples from the same species (i.e., chickpea supplier 1, 2). For example, hemp and lentil were the only two WE proteins that showed similar gelling behavior compared to the DF proteins, even though with variations in the absolute moduli values observed at the end of the gelation process (Table 3). The other WE proteins displayed a less pronounced increase in G′ and G′′, showing a semi-flat pattern in the curve. Notably, soy proteins demonstrated a reduction in both G′ and G′′ values during heating, a trend observed by other authors as well (Jakobson et al., 2023). Finally, the oat protein ingredient collected for this research did not show any rheological variation during the thermal treatment, indicating that it is stable to the heating process. We could hypothesize that this peculiar behavior might be caused by the harsh extraction conditions that formed aggregates not able to form a heat-induced gel as a consequence of their low solubility and insufficient distribution in the system (Yang et al. 2024). Ingredients with heat-stable rheological behavior could be suitable for beverages applications, in which the gel formation is not desired (Sethi et al., 2016). On the other hand, protein ingredients capable of forming a three-dimensional network might find suitability in the preparation of texturized protein (Ma et al., 2022, De Angelis et al. 2023). Consequently, we suggest that a rheological assessment of the protein ingredient is preliminarily carried out to fully unlock its potential use.

Table 3. Mean of final values of G' and G'' of dry-fractionated (DF) and wet-extracted (WE) proteins.

	Sample	G' final (Pa)	G'' final (Pa)
WE	Oat	0.11	0.10
	Chickpea (Supplier 1)	9.25	2.27
	Chickpea (Supplier 2)	54	11
	Lentil	2115	417
	Pea	27	7.82
	Hemp	3563	516
	Soy	58	11
	Wheat gluten	317	42
DF	Chickpea	2554	477
	Lentil (55%)	1158	224
	Lentil (60%)	1859	368
	Pea (55%)	2443	488
	Pea (60%)	4390	863
	Mung bean	1910	353
	Grasspea	2270	425
	Faba bean (55%)	974	186
	Faba bean (65%)	2085	400
	WE	768 ± 1340b	126 ± 212b
	DF	2183 ± 986a	420 ± 195a

Data are expressed as a mean (*n*=3) with a standard deviation <5% of the three technical replicates. Different letters for the same parameter mean significant differences according to the post-hoc Tuckey's HSD test at $p < 0.05$. G' = storage modulus; G" = loss modulus.

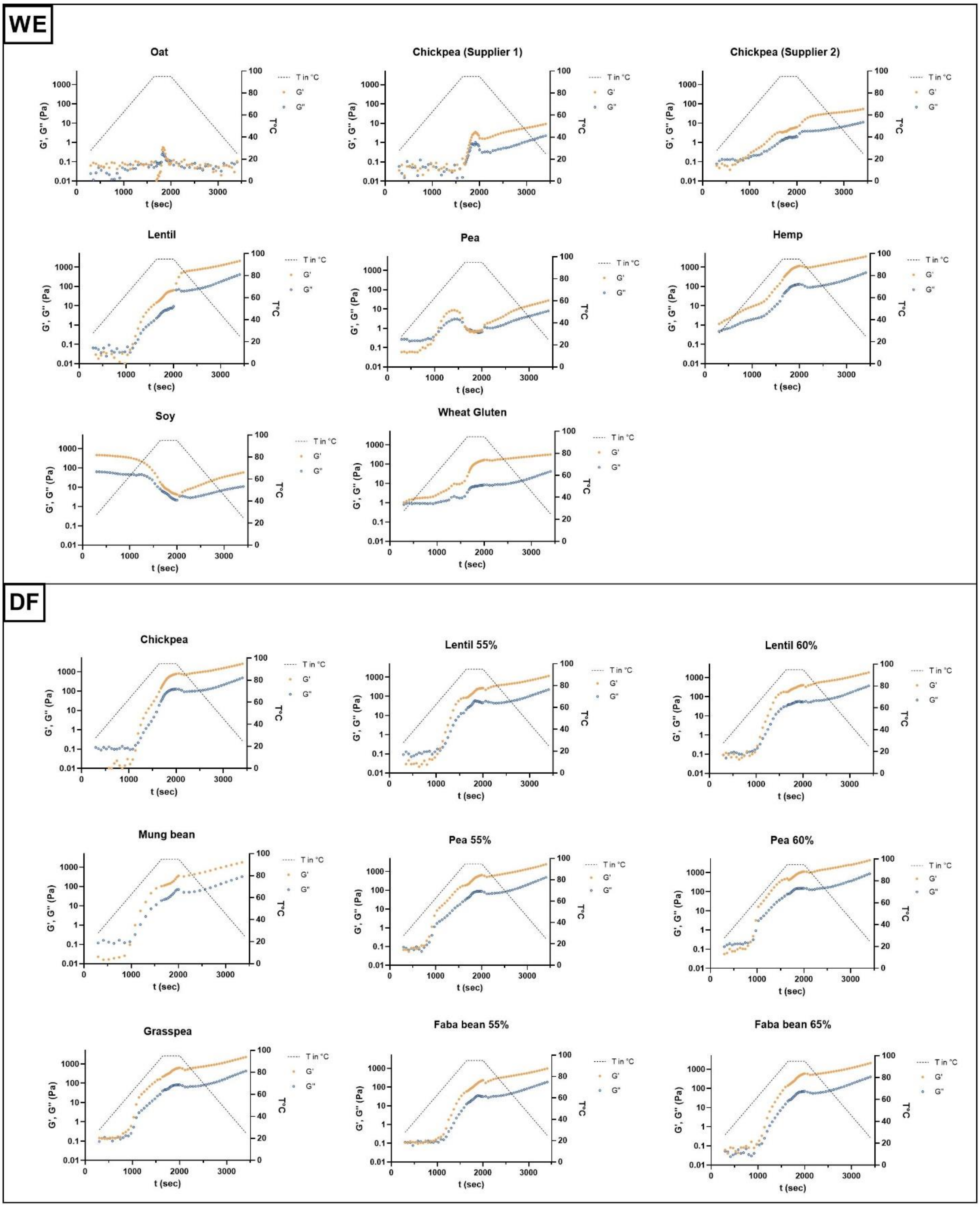


Figure 3. Storage modulus (G', Pa) and loss modulus (G", Pa) of wet-extracted (WE) and dry-fractionated (DF) ingredients dispersions as a function of the temperature (T, °C).

Total, free sulfhydryl group (SH) and disulfide bond (SS) contents

The total, free SH and SS bonds of the protein samples are presented in the Table 4. In general, the comparison between the means of WE and DF protein ingredients displayed significant differences only for the SS bond content, and it can be observed a high data variability among the WE samples (Figure 1). In fact, the free SH content ranged from 4.88 µmol/g for oat protein to 33.05 µmol/g for hemp protein. Total SH content ranged from 32.81 µmol/g for chickpea protein to 68.81 µmol/g for hemp protein. In DF proteins the free SH content ranged from 13.41 µmol/g for faba bean (55%) to 26.80 µmol/g for pea (60%), whereas the total SH content varied from 43.09 µmol/g for faba bean (55%) to 57.52 µmol/g for mung bean. The literature reports similar values for pea, chickpea and lentil protein isolates (Ladjal-Ettoumi et al., 2016), hemp and soy isolates (Xu et al., 2022) and pea protein fractions (Kornet et al., 2021). The content of sulfhydryl groups can vary among samples due to several factors including the amino acid composition (i.e., the presence of sulfur amino acids) and the genotype considered (Ladjal-Ettoumi et al., 2016; Cui et al., 2020; Xu et al., 2022). Moreover, for the purposes of this research, the assessment of the sulfhydryl groups could support the hypothesis discussed in the next paragraphs concerning the conformational structure of the protein (Gao et al., 2020; Chang et al., 2022). Sulfhydryl groups are influenced by the extraction procedures (Jiang et al., 2017; Cui et al., 2020; Gao et al., 2020) and they are involved in the reactions caused by protein unfolding during heat-induced gelation process, while disulfide bonds contribute to the formation of strong gels (Yang et al., 2021). It has been previously reported that high-alkaline conditions during extraction (e.g., alkaline extraction and isoelectric precipitation process) can result in the cleavage of disulfide bonds and in an increase of free SH, that can be considered an indicator of conformational changes in the protein structure (Gao et al., 2020). Therefore, a higher free sulfhydryl group content may be related to the exposure of internal groups due to protein unfolding together with the breakdown of disulfide bonds (Cui et al., 2020; Gao et al., 2020). This trend was not clearly visible in our samples, also considering that the differences detected were not significant, because of the high variability within the class of proteins. For instance, if the oat protein were excluded from the data analysis – because of its very low content of free SH groups compared to the other proteins – the differences in free SH would become significant (data not shown). This might confirm the hypothesis that WE proteins were subjected to denaturation during extraction (possibly confirmed by the high free SH content), corroborating what was previously discussed with the techno-functional properties and the rheological behavior.

Table 4. Free and total sulfhydryl groups (SH), and disulfide bonds (SS) of dry-fractionated (DF) and wet-extracted (WE) proteins.

		Total SH (µmol/g)	Free SH (µmol/g)	SS bonds (µmol/g)
WE	Oat	32.91	4.88	14.02
	Chickpea (Supplier 1)	32.81	19.63	6.59
	Chickpea (Supplier 2)	39.59	21.85	8.87
	Lentil	37.97	25.26	6.36
	Pea	51.82	30.18	10.82
	Hemp	68.81	33.05	17.88
	Soy	51.68	20.10	15.79
	Wheat gluten	55.47	21.55	16.96
DF	Chickpea	46.65	19.13	13.76
	Lentil (55%)	52.51	16.35	18.08
	Lentil (60%)	53.40	14.70	19.35
	Pea (55%)	46.92	20.28	13.32
	Pea (60%)	54.54	26.80	13.87
	Mung bean	57.52	21.45	18.04
	Grasspea	56.36	20.78	17.79
	Faba bean (55%)	43.09	13.41	14.84
	Faba bean (65%)	44.86	15.96	14.45
	WE	46.38 ± 12.68a	22.06 ± 8.47a	12.16 ± 4.62b
	DF	50.65 ± 5.32a	18.76 ± 4.14a	15.94± 2.33a

Data are expressed as a mean (*n*=3) with a standard deviation <5% of the three technical replicates. Different letters for the same parameter mean significant differences according to the post-hoc Tuckey's HSD test at $p < 0.05$.

Exploratory analysis on the secondary structure of proteins

The FTIR spectra acquired from WE and DF samples are shown in Figure 4, along with the preprocessed spectra using the Savitzky-Golay second order derivation, which facilitated the extrapolation of hidden peaks representative of different protein structures, and the PCA results.

The group of frequencies between 1600 and 1700 cm^{-1} is identified as the Amide I region, characterized by the C=O stretching vibration of the amide groups in conjunction with the in-phase bending of the N-H bond and stretching of the C-N bond (Yang et al., 2015). The literature reports wavelength ranges associated with each structure, but the peak association is often inconsistent. In general, extended chains or intermolecular β-sheet can be observed at 1600-1615 cm^{-1} (Tang et al., 2021) or 1610-1627 cm^{-1} (Nasrollahzadeh et al., 2023); wavelength of about 1620-1642 cm^{-1} (Tang et al., 2021, Chang et al. 2022, Nasrollahzadeh et al., 2023) corresponds to β-sheet structures. Carbonaro et al. (2011) studied the secondary structure of different types of food proteins and reported that β-sheet structures can be assigned to the bands 1630-1638 cm^{-1}, assuming the existence of both parallel and antiparallel β-sheets within this region. Random coils and α-helix are found at about 1640-1650 cm^{-1} (Tang et al., 2021, Nasrollahzadeh et al., 2023) and 1650-1659 cm^{-1} (Tang et al., 2021, Chang et al., 2022, Nasrollahzadeh et al., 2023), respectively. β-turn structures are found at 1660-1680, 1694 cm^{-1} (Tang et al., 2021), 1670 cm^{-1} (Chang et al., 2022), 1660-1699 cm^{-1} (Nasrollahzadeh et al., 2023). Finally, anti-parallel β-sheet and aggregates can be found at 1680 cm^{-1} and 1692 cm^{-1}, respectively (Chang et al., 2022). The primary objective of this analysis is to elucidate the differences between the two groups of proteins, and the data are discussed based on exploratory analysis, observing the results of PCA (Figure 4). Principal Component (PC) 1 explains most of the variance (74.22%) and enables a clear distinction between the two groups of proteins. From the score plot, it is evident that DF proteins are located in the negative part of PC1, whereas all the WE proteins exhibit positive scores on PC1. Additionally, as confirmed by the other analytical determinations, WE proteins showed higher variability compared to DF ones. The analysis of the loading plot, which demonstrates the contribution of each wavelength to PC1 and PC2, reveals that the region between 1613 and 1622 cm^{-1} has negative scores, therefore characterizing DF proteins. According to the literature, this region may be influenced by the presence of intermolecular chains (Carbonaro et al., 2011; Tang et al., 2021; Nasrollahzadeh et al., 2023). By contrast, a positive contribution to PC1 is given by the regions between 1631-1646 cm^{-1}, representative of β-sheet and random coil structures (Tang et al., 2021). Consequently, WE proteins appeared to be richer in these structures compared to DF ones. Some possible explanations were hypothesized to discuss the results. First, a higher content of random coil structure is expected due to protein denaturation occurring during wet extraction procedures (Zhu et al., 2010; Yang et al., 2021). Beck et al. (2017) suggested that a heat treatment can cause an increase in β-sheet structures. It should be also considered that defatting may cause changes in the protein structures (Yue et al., 2021). Moreover, the globulin fraction is the most abundant in WE proteins (Yang et al., 2021) and the 7S globulins are particularly rich in β-sheet structures (Carbonaro et al., 2011; Chang et al., 2022). Finally, other bands associated with α-helix and β-turn seem to be not influent on PC1. Unfortunately, data and studies on the secondary protein structure of dry-fractionated proteins are scarce, and the effect of the dry fractionation technology could be the subject of future research. This is particularly important because the secondary structure of protein can further explain some techno-functional properties such as protein solubility (Cui et al., 2020). In fact, Cui et al., (2020) hypothesized that the high content of β-turn structures might contribute to a low solubility in yellow pea protein. However, the same authors noted that such findings are not well comprehended in the literature, which often reports conflicting results (Cui et al., 2020). In addition to providing information about the secondary structure of proteins, FTIR analysis allows us to clearly distinguish between DF and WE proteins, suggesting the usefulness of this analytical approach for authentication purposes. In fact, food authentication is a modern and challenging objective in the food sector, and the possibility of applying rapid and non-destructive methods is a key topic in the current food research (De Angelis et al., 2024c; https://agritechcenter.it/spokes/). For example, FT-NIR has been successfully applied for the detection of adulterants in proteins such as soy, whey (a source of lactose), and wheat (a source of gluten) (Neves et al., 2022). The use of DF proteins in food formulation allows for the development of foods with distinctive characteristics, such as low-processed or clean label products. Ma et al., (2022) reported that protein ingredients can also be labeled as 'flour' when the protein content is near 50%, offering advantages in labeling. Therefore, the detection of DF proteins could be an interesting subject for further studies.

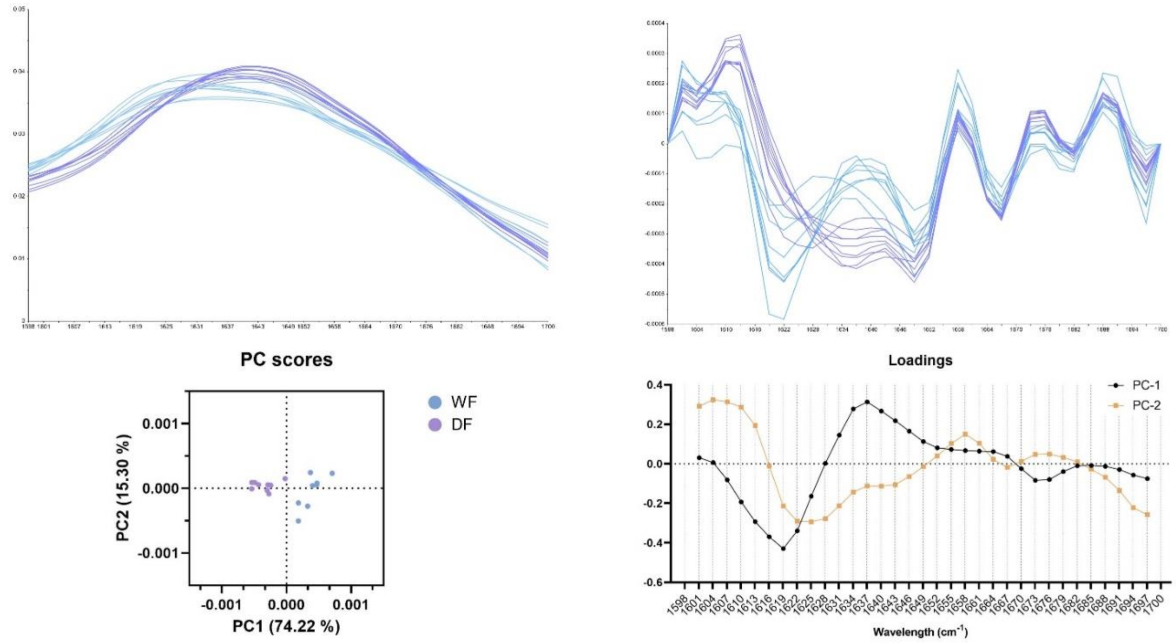


Figure 4. Results of the exploratory analysis of the secondary structure. Top left: raw FTIR spectra within the amide 1 region; top right: processed spectra using the Savitzky-Golay second order derivation and two smoothing points; bottom left: loading plot of the Principal Component Analysis carried out on the preprocessed spectra; bottom right: score plot of the Principal Component Analysis.

Conclusions

The results of this research indicated a clear distinction between the protein ingredients obtained with wet extraction and dry fractionation, confirming the significant impact of the extraction technologies on techno-functional and chemical properties of the ingredients. In particular, data suggested that wet extraction technologies can lead to ingredients with techno-functional properties typically associated with a denatured protein structure. By contrast, the protein ingredients produced using DF processes showed the highest WSI, protein solubility at pH 7 and 9. Moreover, FC and FS and EC, were higher in DF proteins compared to WE ones, and this was principally linked to the non-denatured protein structures of the ingredients and to the presence of other non-protein constituents that enhanced their techno-functional properties. The analysis of the gelling properties demonstrated how ingredients obtained with the two processes can have different behaviors, which can be exploited for the development of specific food products. The results of the techno-functional characterization were confirmed by the chemical determinations. For instance, the highest presence of disulfide bonds in the DF proteins, together with the differences detected in the secondary structure of proteins, support the hypothesis that wet extraction led to important conformational and compositional changes and, consequently, to very different techno-functional properties. While the modulation of functionality provided by wet extraction methods can be advantageous for tailoring food applications, the important techno-functional properties associated with a non-denatured protein structure and a complex composition have emerged. This would also support the transition toward a more sustainable food system, considering that proteins produced with dry fractionation technologies have a lower environmental footprint compared to proteins obtained with wet extraction processes.

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Author contributions

Davide De Angelis: Conceptualization, Investigation, Formal analysis, Writing - Original draft preparation, Writing - Review & Editing; Vittoria Latrofa: Conceptualization, Investigation, Formal analysis, Writing - Original draft preparation; Giacomo Squeo: Conceptualization, Resources, Writing - Review & Editing; Antonella Pasqualone: Conceptualization, Writing - Review & Editing; Carmine Summo: Conceptualization, Resources, Project administration, Writing - Review & Editing.

Conflict of Interest: none

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Chapter 3. Dry-fractionated protein concentrate as egg replacer in sponge cake: how the rheological properties of the batters affect the physical and structural quality of the products

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Abstract

Egg replacement is a food trend worthy of the attention of both academics and industry. Dry-fractionated protein concentrates (DFp) are minimally processed and sustainable ingredients. DFp from chickpea, red lentil and mung bean, in the form of aqueous dispersions at 20, 30, and 40% (w/w) were used to replace egg in sponge cakes. To gain information about the effect of DFp on the physico-chemical features of the sponge cakes, the rheological properties (i.e., flow behavior, frequency-dependent and temperature-dependent behaviors) of the batters were investigated.

Frequency sweep revealed a storage modulus (G') higher than loss modulus (G''), indicating the predominance of an elastic-like behavior, depending on the frequency. Higher DFp amount conferred a firmer structure to the batters, as indicated by higher apparent viscosity when 40% DFp were used. During temperature sweep, G' increased starting from 80 °C in all DFp-based batters, indicating protein and starch conformational changes. Higher DFp content better simulated the egg behavior in terms of specific volume and thickness variation after baking, but it led to harder cakes. Crumb structure was similar to the control, highlighting that the DFp can imitate the egg behavior in cake preparation. Protein content of the cakes containing 30% DFp was similar to the control. However, the addition of DFp caused the phytic acid increase. Chickpea and lentil DFp had a lower impact on the sensory properties compared to mung bean.

Overall, eggs in cakes formulation can be substituted by plant-based protein produced by dry fractionation. However, further studies are needed to evaluate nutritional characteristics.

Keywords

Egg replacers; dry-fractionated protein; rheological properties; sponge cake batter; crumb structure; plant-based protein.

Introduction

The popularity of plant-based proteins is steadily growing, due to increased awareness of health and sustainability issues related to the production and consumption of animal-based proteins (Yazici and Ozer, 2021). In most cases, the plant proteins available on the market, and then used to prepare food, are obtained by a "wet fractionation" procedure, which involves the use of water together with alkali, acids, and/or other chemical substances to extract the protein (Schutyser et al., 2015). The conditions used in this process, e.g., the acidic environment and/or the temperatures used in the drying operations, generally cause a protein denaturation, leading to the loss of some functional properties, such as the solubility, the foaming ability, and the gelling capacity (Vogelsang-O'Dwyer et al., 2020). By contrast, the "dry fractionation" process requires less resources, as it concentrates proteins by relying exclusively on physical methods (De Angelis et al., 2021). Moreover, the dry fractionation process preserves the protein structure, resulting in a better protein functionality, especially regarding the foaming ability and foam stability (Vogelsang-O'Dwyer et al., 2020).

Such functional properties of the dry-fractionated protein are particularly suitable to formulate sponge cakes or similar bakery products which require the incorporation of air and the retention of gas cells in the cake matrix (Pycarelle et al., 2020). Traditionally, this structuring and stabilizing role is played by the egg proteins, which then have a major effect on the quality of the cake. However, consumer concerns related to the presence of cholesterol, the allergenicity of the egg proteins, as well as the expanding demand for plant-based food (Yazici and Ozer, 2021) triggered an increasing interest in finding egg replacer alternatives (Yazici and Ozer, 2021). In fact, the egg replacer market is expected to grow at a Compounded Average Growth Rate (CAGR) of 5.61% from 2022 to 2028 (Mordor Intelligence, 2023).

According to a recent review by Yazici and Ozer (2021), legume protein ingredients, especially soy protein, are frequently used as egg replacers. In addition, hydrophobically modified pea protein isolate (Shah et al., 2019), aquafaba (Nguyen and Tran, 2019) and lentil protein isolate (Jarpa-Parra et al., 2017) have been previously proposed as egg substitutes in cake formulations.

Chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* (L.) Medik.) are typical legumes of the Mediterranean area and they were previously studied for their performance during the dry fractionation process (De Angelis et al., 2021; Pelgrom et al., 2015). Mung bean (*Vigna radiata* (L.) R. Wilczek.) is widely produced and consumed in Asian countries and is gaining interest worldwide due to its nutritional (Hou et al., 2020) and for the functional properties of its dry-fractionated protein concentrates (Schlangen et al., 2022). However, to the best of the authors' knowledge, the utilization of dry-fractionated proteins as egg replacers in cake formulation has not been previously reported.

Therefore, in this paper dry fractionated proteins obtained from chickpea, lentil and mung bean were used as egg replacer in sponge cake preparation with the aim of investigating their effect on the rheological properties of the batters and on the textural properties and the physico-chemical quality of the sponge cakes.

Materials and methods

Materials

Dry-fractionated chickpea, mung bean and lentil protein concentrates were purchased from InnovaProt s.r.l. (Gravina in Puglia, Italy), and they had 56.8 56.9 and 55.7 g/100g protein content (dry matter basis) respectively, and 7.3, 2.6 and 2.5 g/100 g lipid content (dry matter basis) respectively. The protein and lipid content were determined as described in the paragraph 2.6.

Plain wheat flour (type "00" Molino Casillo Spa, Corato, Italy), granulated sugar (Eridania, Bologna, Italy), corn starch (Maizena, Rome, Italy), whole chicken eggs (Aia S.p.A, Caselle, Italy), almond drink (Abafoods Srl, Badia Polesine, Italy), sunflower oil (Olearia De Santis, Bitonto, Italy) and baking powder (Cameo S.p.A., Desenzano del Garda, Italy) were purchased from the local market.

Preparation of sponge cakes

Sponge cakes were prepared according to Majzoobi et al. (2014) with some modifications. The formulation of the sponge cakes is reported in Table 1. To prepare the egg replacer, water and dry-fractionated protein concentrates (DFp) of lentil (L) chickpea (C) and mung bean (M) at ratios of 80:20, 70:30 and 60:40 (w/w) were whipped for 12 min with an electric mixer at 2300 rpm (5KHM5110EWH, Kitchen Aid, Antwerp, Belgium). The sugar was then added and whipped for 3 min. For the preparation of the control sponge cake (CTRL), eggs and sugar were whipped following the same process. Then, the liquid ingredients i.e., the almond drink and the sunflower oil were added, and mixed for 7 min at 100 rpm. Finally, the wheat flour, the corn starch and the baking powder were added and mixed for 7 min at 100 rpm. The batter was poured into springform cake pans having a diameter of 10 cm, and baked in an electric oven (Smeg, Guastella, Italia) for 35 min at 180 °C following a Latin square design pattern to minimize any effect of the location during baking. The sponge cakes were allowed to cool at room temperature for 2 h and then analyzed.

Table 1. Formulation of the sponge cake control and sponge cake with egg replacer.

Ingredients (g)	C20	C30	C40	L20	L30	L40	M20	M30	M40	CTRL
Eggs	-	-	-	-	-	-	-	-	-	100
Chickpea protein concentrate	20	30	40	-	-	-	-	-	-	-
Lentil protein concentrate	-	-	-	20	30	40	-	-	-	-
Mung bean protein concentrate	-	-	-	-	-	-	20	30	40	-
Water	80	70	60	80	70	60	80	70	60	-
Other ingredients for each trial*	430									

Other ingredients for each trial*: sugar (80 g), corn starch (40 g), flour (160 g), baking powder (10 g), sunflower oil (40 g), almond milk (100 g).

Rheological properties of batters

The rheological properties of batters were measured with a rheometer HAAKE MARS iQ Air (Thermo Fisher Scientific, Waltham, Massachusetts, USA) equipped with a parallel plate geometry (P35/Ti-02180932). All the determinations were carried out at 1 mm gap between the plates.

The shear rate ramp test was performed by increasing the shear rate from 1 to 100 1/s at 25 °C. The data were fitted to the Ostwald–de Waele model, according to the following equation (Tsatsaragkou et al., 2021):

$$\tau = K \dot{\gamma}^n$$

were: τ = shear stress (Pa), K = consistency index (Pa s^n); $\dot{\gamma}$ = shear rate (1/s), and n = flow behavior index.

The oscillatory frequency sweep and temperature sweep were carried out following the procedures described in Li et al. (2022) with some modifications. For the oscillatory frequency sweep the frequency varied from 0.1 to 10 Hz, at strain value of 0.5%, within the linear viscoelastic region of the batters. The storage modulus (G') and loss modulus (G") as a function of the frequency were recorded. The loss factor (tan δ) was calculated as the ratio between G˝ and G′ (Schlangen et al., 2022).

The temperature sweep test was performed at a 0.5% strain and 1 Hz frequency. The batters were heated from 25 °C to 95 °C at a heating rate of 5 °C/min. Then, they were kept at 95 °C for 5 minutes. The geometry containing the sample was covered with a sample hood during the analysis to prevent the water evaporation. The G' and G'' moduli were recorded as a function of the temperature. Two technical replicates for each batter were performed.

Physical and textural properties of the sponge cake

The specific volume of sponge cakes (mL g^{-1}) was determined following the rapeseed displacement method as described in the method 10-05.01 (AACC, 2009).

The thickness variation after baking was evaluated with a caliper as described in Pasqualone et al. (2020) and calculated as follows:

$$\% \text{ of variation of thickness} = \frac{\text{thickness after baking } - \text{thickness before baking}}{\text{thickness before baking}} \times 100$$

The texture profile analysis (TPA) was performed using a Z1.0 TN texture analyzer (Zwick Roell, Ulm, Germany) equipped with a cylindrical probe (36 mm diameter) and a 50 N load cell, according to the method described in Gómez et al. (2021) with some modifications. The samples were cut into cubes (25 mm side) and compressed twice at 1 mm s^{-1} speed until 50% deformation was reached, with 5 s pause between the two compressions. The following parameters were evaluated, according to Summo et al. (2020): firmness (N), indicating the maximum force recorded during the first compression; springiness, measured by the height detected during the second compression divided by the original height of the sample; cohesiveness, measured as the area of work during the second compression divided by the area of work during the first compression; chewiness (N), calculated as firmness × cohesiveness × springiness.

The image analysis of the crumb structure was carried out with the method described in De Angelis et al. (2020) using ImageJ software (National Institutes of Health, Bethesda, USA). Briefly, sponge cakes were cut in half, and an RGB image was collected by a Canon EOS 600D camera (Canon, Tokyo, Japan) equipped with a Canon EF-S 18-200mm f/3.5-5.6 IS lens. The image was first converted into 8-bit grayscale, and an area of 40 × 40 mm was considered for the analysis. The threshold function was used to process the image and carry out the cell count, selecting cells having an area ≥0.05 mm^2. The cell density was calculated as the number of cells divided by the area used for the analysis.

Chemical determinations

Protein content (N × 6.25) of the dry-fractionated proteins and sponge cakes were determined according to the method AOAC 979.09 (AOAC, 2006). The lipid content was determined by a Soxhlet apparatus (SER 148 extraction system, Velp Scientifica srl, Usmate, Italy) following the method AOAC 945.38F (AOAC, 2006), using diethyl ether as extraction solvent. Phytic acid was determined using the phytic acid assay kit K-PHYT 05/07 (Megazyme, Bray, Ireland), in accordance with the instructions of the manufacturer.

Sensorial analysis

Quantitative Descriptive Analysis (QDA) of sponge cakes was performed by a panel of 9 trained members composed of 4 men and 5 women (age between 26 and 57 years). Panellists were informed on the objectives of the study, and each participant provided written informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They indicated that did not suffer from any food intolerances and allergies. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption. A training session to acquire confidence with the lexicon was carried out, to fix the scale anchors, define the list and the intensity of the descriptors as described in Pasqualone et al. (2019). The panelists evaluated the sponge cakes using an unstructured 10 cm scale in terms of crust color (i.e., 0: yellow – 9: brown) and crumb color (i.e., 0: white – 9: dark yellow), sweetness, legume flavor and astringency (i.e., 0: not perceived – 9: very intense).

Statistical analysis

Two different batters for each formulation were prepared and the determinations on the batters were repeated two times (*n*=2). For each batter two different baking trials have been performed (*n*=4). All the experimental data of the trials containing the egg replacer were subjected to analysis of variance two-way ANOVA followed by the Tukey's HSD test for multiple comparisons at $\alpha = 0.05$, considering the amount of DFp and the type of legume as independent variables, together with their interaction. The differences between the samples and the control were evaluated using Dunnett's multiple comparisons test at $\alpha = 0.05$. The data on the physical and textural properties of the sponge cake were also subjected to a Pearson correlation at 95% confidence interval. All data were processed by Minitab Statistical Software (Minitab Inc., State College, PA, USA).

The plot of the rheological data and of the sensory properties (i.e., an heatmap) were made using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA).

Results and discussion

Rheological properties of batters

Frequency sweep analysis and flow behavior of the batters

The storage modulus (G') and loss modulus (G") of the batters as a function of the frequency (Hz) are depicted in Fig. 1. They represent the total resistance of the batter to flow as a function of the linear frequency. All the batters containing the DFp exhibited a storage modulus (G') higher than loss modulus (G''), indicating the predominance of an elastic-like behavior (Matos et al., 2014), with a dependence on the frequency. In fact, both moduli increased with the increase of the frequency, and this behavior was previously reported in cake batters supplemented with vegetable and animal proteins (Matos et al., 2014; Bustillos et al., 2020). The CTRL batter showed a frequency-dependent behavior similar to the batters containing the DFp. However, the absolute values of the moduli were lower than in the other samples, indicating a batter with a more fluid-like behavior. Moreover, the moduli were nearly overlapped, especially at high frequency, reaching the cross-over point at 10Hz, confirming a more liquid than elastic behavior of the CTRL batter compared to the DFp-based batters (Bustillos et al., 2020; Yang et al., 2022). This is also evidenced by the tan δ calculated at 1 Hz for all the batters and reported in Table 2. In particular, the CTRL batter showed the highest tan δ, which indicates a more liquid-like behavior, without significant similarities with any of the batters containing DFp. Therefore, DFp conferred a more elastic solid-like behavior to the batters, and this effect was clear by observing flow curves (apparent viscosity as a function of the shear rate) shown in Fig. 2. The batters containing the 40% of DFp showed the highest viscosity, and they were consequently characterized by the highest consistency index (K) calculated by the Ostwald–de Waele model (Table 2). The hardening effect of the vegetable protein was previously observed (Matos et al., 2014; Shevkani et al., 2014) and it can be related to the water absorption of the protein and to a reduction of free water in the batter cake (Jarpa-Parra et al., 2017). Moreover, Bustillos et al. (2020) reported that the viscosity and K in cake batter increased at higher concentration of pea protein because protein particles tended to connect to each other in the continuous phase of the batter.

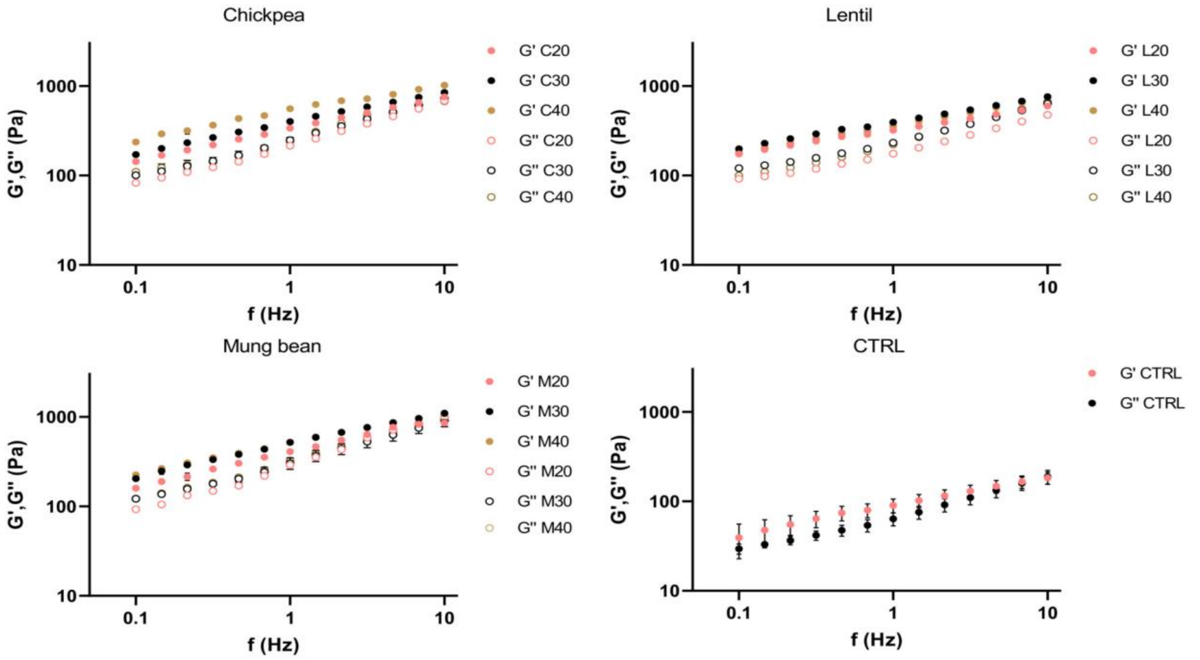

Figure 1. Mean storage modulus (G', in Pa) and loss modulus (G'', in Pa) of the sponge cake batters prepared with the aqueous dispersion of chickpea (C), lentil (L), and mung bean (M) proteins at 20, 30, and 40 % as a function of the regular frequency (f, in Hz). The bars indicate the standard deviation (*n* = 2). CTRL: control sponge cake batter.

Furthermore, the batters showed a shear thinning behavior, having a flow behavior index (n) lower than 1 (Table 2), suggesting that the batter microstructure aligns into the flow direction as the shear rate increases, causing a decrease of the apparent viscosity (Jarpa-Parra et al., 2017). The viscoelastic properties of cake batter affect the quality features of the final product, such as the volume, the crumb porosity, and the texture (Yazici and Ozer, 2021).In particular, during the baking process, a lower batter viscosity can easily raise the bubbles to the surface and cause them to lose in the atmosphere, resulting in cakes collapse in the oven (Shevkani et al., 2014). On the contrary, if the consistency of the batter is too high it might hamper the expansion of air bubbles during baking (Yazici and Ozer, 2021), therefore, compromising the quality of the product.

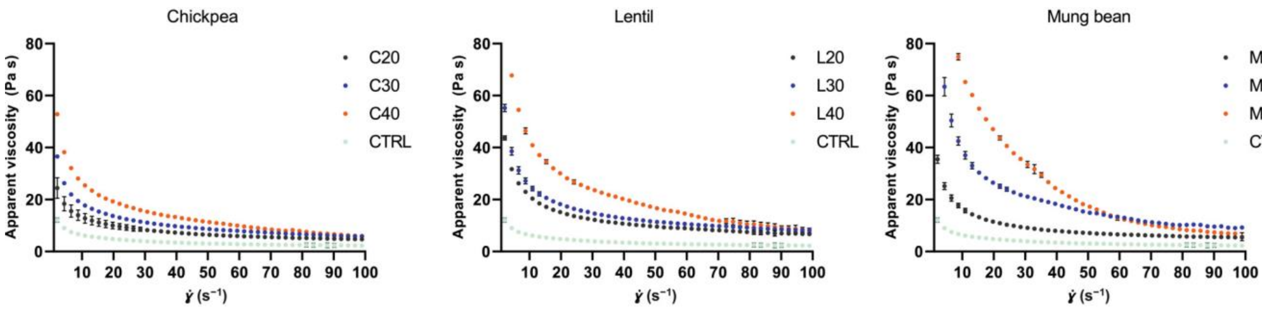

Figure 2. Apparent viscosity (Pa s) of the sponge cake batters prepared with the aqueous dispersion of chickpea (C), lentil (L), and mung bean (M) proteins at 20, 30, and 40 % as a function of the shear rate ($\dot{\gamma}$, in 1/s). The bars indicate the standard deviation (*n* = 2). CTRL: control sponge cake batter.

Viscoelastic properties of the batters during heating

The temperature dependent behavior of the batters is depicted in Fig. 3. The temperature sweep was used to simulate and understand the main rheological phenomena occurring during the baking process. The trend observed was similar in the batters containing the egg replacer prepared with the three legumes under investigation. In the early stage of the analysis, the G' remained almost constant with small fluctuations. The latter were probably given by the temperature increase and by the heat transfer in the batter, resulting in changes in the viscosity of the product. A sharp increase of G' was then observed at about 80 °C in all the formulations containing the DFp, and it continued until 95 °C at the same rate.

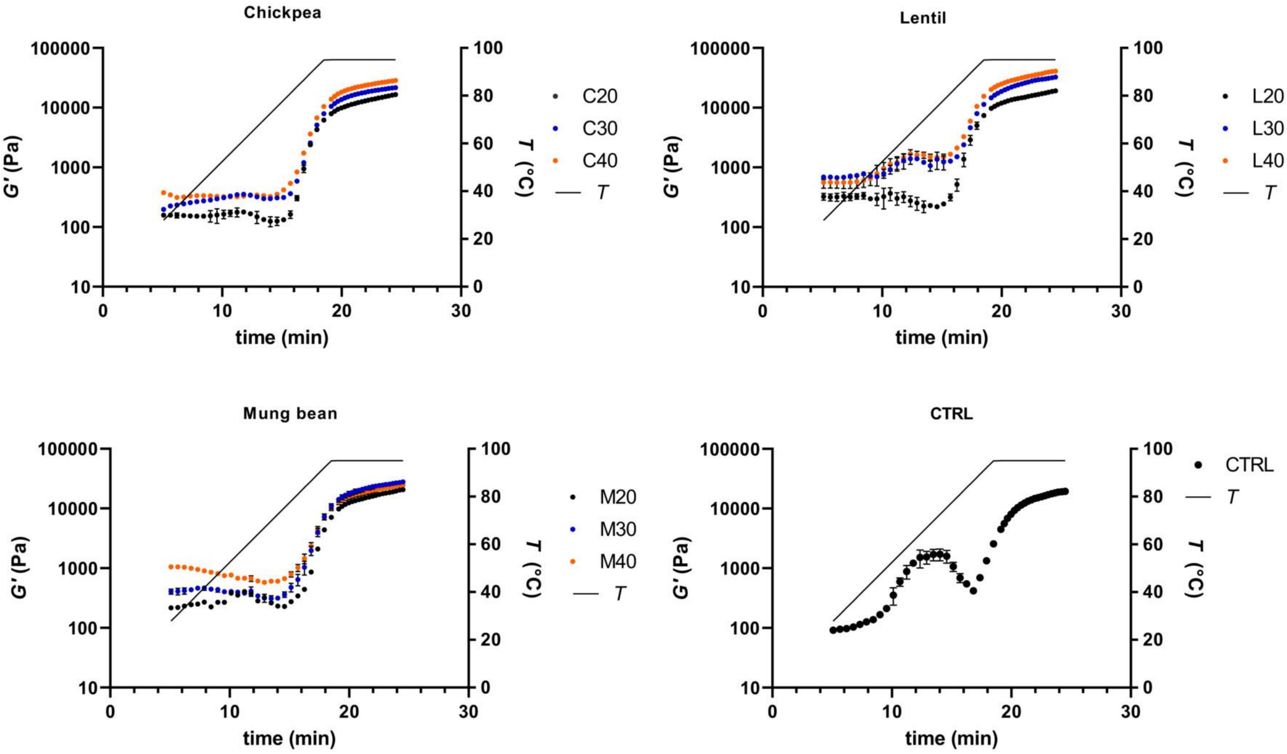

Figure 3. Storage modulus (G', in Pa) of the sponge cake batters prepared with the aqueous dispersion of chickpea (C), lentil (L), and mung bean (M) proteins at 20, 30, and 40 % as a function of the temperature (T, in °C). The bars indicate the standard deviation (*n* = 2). CTRL: control sponge cake batter.

During the isotherm step at 95 °C, G' slightly increased in all the formulations. This behavior explains the conformational changes due to the protein denaturation, coagulation, and the subsequent gelation, together with the starch gelatinization (Alvarez et al., 2017). Similar heating patterns were observed in previous studies on batters formulated with legume proteins (Matos et al., 2014; Alvarez et al., 2017). In particular, it was previously reported that the globulin contained in the legume proteins denatures at a temperature range of 78-83 °C, causing an increase of the G' modulus (Guldiken et al., 2021; Schlangen et al., 2022). Therefore, it is reasonable to assume that the structure of the product started to develop at about 80 °C, changing from a fluid, aerated emulsion, to an elastic and porous structure (Tsatsaragkou et al., 2021). With this respect, the temperature sweep tests can be considered an excellent tool to understand the structural modifications in the batters during a heating process, giving information on the viscoelastic changes during the cooking process (Alvarez et al., 2017).

Table 2. Rheological properties of the cake batters prepared with the aqueous dispersion of chickpea (C), lentil (L), and mung bean (M) proteins at 20, 30, and 40 %. K: consistency index; n: flow behavior index. CTRL: control sponge cake batter.

	Flow behavior (shear rate ramp)		Frequency sweep at 1 Hz			Temperature sweep at 95°C
Sample	K (Pa s^n)	n	G' (Pa)	G'' (Pa)	tan (δ)	G’ (Pa)
C20	40.62 ± 10.63b	0.54 ± 0.06a	338 ± 2c	215 ± 2c	0.64 ± 0.00b	16673 ± 794c
C30	57.89 ± 6.31b	0.52 ± 0.03a	400 ± 8b	247 ± 11b	0.62 ± 0.02b	21714 ± 735b
C40	128.65 ± 10.55a	0.36 ± 0.02b	667 ± 22a	447 ± 18a	0.67 ± 0.00a	29192 ± 712a
L20	69.58 ± 7.93b	0.49 ± 0.04a	320 ± 16c	176 ± 9b	0.55 ± 0.00a	20415 ± 1285c*
L30	79.85 ± 8.38b	0.51 ± 0.03a	393 ± 5a	232 ± 7a	0.59 ± 0.01a	32510 ± 100b
L40	260.00 ± 33.70a	0.28 ± 0.04b	356 ± 6b	223 ± 26a	0.63 ± 0.08a	41035 ± 1485a
M20	45.85 ± 1.59c	0.53 ± 0.00a	408 ± 7b	290 ± 2a	0.58 ± 0.01a	20730 ± 470b*
M30	124.55 ± 1.45b	0.46 ± 0.00b	517 ± 23a	325 ± 10a	0.54 ± 0.01b	27920 ± 670a
M40	242.25 ± 9.05a	0.46 ± 0.01b	520 ± 20a	303 ± 32a	0.55 ± 0.00b	25718 ± 1338a
CTRL	18.06 ± 0.72	0.54 ± 0.01	90 ± 11	64 ± 7	0.71 ± 0.00	19480 ± 283

Different letters for the same parameter mean significant differences within the same species, according to the Tuckey’s HSD test ($p \leq 0.05$). * the value is similar to the control (CTRL) according to Dunnett’s test ($p > 0.05$). $n = 2$

More in depth, it is possible to note some significant effect on G’ modulus recorded at the end of the analysis (Table 2), that are particularly related to the protein concentration and to the protein type. It is evident that increasing the protein concentration led to a significant increase of the G’ in all the three proteins under investigation, indicating that the proteins were responsible for strengthening the elastic behavior of the batter. Moreover, considering the absolute values, lentil-based batters showed the highest G’. This might be related to the protein fractions which are mainly composed of legumin-like and vicilin-like globulins and albumin (approximately 15%). Lentils contain a slightly higher quantity of globulins (approximately 70%), compared to mung bean (approximately 62%) (Shrestha et al., 2022) and chickpea (approximately 53-60%) (Grasso et al., 2022). Therefore, such differences may explain the G’ values, being the globulin fraction predominantly involved in the gelation reactions (Grasso et al., 2022). Moreover, G’ values of L20 and M20 were significantly similar to the CTRL batter. During heating, the CTRL batter showed two inflection points at 45-50 °C and at about 85 °C, with a short drop in the G’ modulus observed between 80 and 85 °C. This trend was previously observed in control batters prepared with wheat flour and whole eggs (Tsatsaragkou et al., 2021), and it was explained by a drop in the viscosity due to the sugar dissolution in the batter (Tsatsaragkou et al., 2021). The higher consistency of the batters containing the egg replacer might have hidden this drop, mitigating the effect of the sugar dissolution. The mechanism of the structure formation of egg-based cakes during baking has been reported in a recent review (Hedayati et al., 2022), and it majorly involves the denaturation of ovalbumin contained in the egg white and the coagulation of the egg yolk proteins (livetin, phosvitin, and lipoproteins), which contribute to the network development through the formation of disulfide bonds and other intermolecular interactions (Hedayati et al., 2022). Overall, despite the differences in the protein involved, the development of consistency during the thermal treatment showed similarities between the batter containing the egg replacer and the CTRL, indicating that the DFp are able to imitate the egg viscoelastic behavior when used in cake batters.

Physical and textural properties of the sponge cake

The physical and textural properties of the sponge cakes are reported in Table 3. The variables "type of legume" significantly influenced all the parameters under investigation, except for the cell density, whereas the variable DFp amount was not significant only for the cell class $0.5 < x < 1$ mm^2. Moreover, a significant interaction between species and concentration was found for all the physical properties of the cakes, except for the cell class $0.5 < x < 1$ mm^2 (p=0.056), meaning that the magnitude of the changes observed in the parameters were not solely dependent on the species or the concentrations (main effects), but also on their combinations. In particular, considering the specific volume, it appeared to be positively affected by the quantity of DFp used in the sponge cake formulation, with the highest values found for the sponge cakes containing 40% of DFp. The species also affected the specific volume, and the sponge cakes produced with the lentil DFp showed the highest specific volume, followed by the chickpea-based and mung bean-based products. The CTRL sponge cake showed a specific volume of 2.4 mL/g which was only comparable to the L40 sponge cake. The other trials showed a lower specific volume compared to the CTRL, indicating that a lower protein concentration led to a less expanded structure. It was recently reviewed that the specific volume is highly variable in cakes formulation, ranging from 1.38 to 4.08 mL/g (Hedayati et al., 2022) therefore the results obtained with the formulations proposed in this study fall in this range. The specific volume, being dependent on the ability of the cake batter to retain gases during the baking process, is a direct indicator of the quality of the sponge cakes (Yazici and Ozer, 2021; Lu et al., 2023). In particular, the specific volume could be related to both batter viscosity and to the gel-forming ability of batter during baking (Yazici and Ozer, 2021). In fact, the observed results are corroborated by the rheological properties of the batters, that highlighted the highest apparent viscosity (Fig. 2) and the highest G' after the heat treatment (Fig. 3) in the batters containing 40% DFp. Therefore, higher quantity of DFp might have improved viscoelastic properties of the batters, also enhancing the retention of the gases developed during baking (Jarpa-Parra et al., 2017; Shevkani et al., 2014), without hamper the expansion of air bubbles. Consequently, a similar trend was observed with the thickness variation recorded after baking, as also demonstrated by the significant and positive correlation with the specific volume (r=0.753, p=0.011). Therefore, it could be assumed that high DFp concentrations are needed to replicate the expansion phenomena given by egg in cakes preparation.

The results of the texture profile analysis of the sponge cakes are also reported in Table 3. Both species and concentration significantly influenced the textural properties of the cake. In fact, the variable "type of legume" was highly significant for all the textural properties ($p \leq 0.001$). In particular, the lentil protein conferred a stronger structure to the products compared to the other proteins. Overall, the lentil-based cakes showed the highest firmness and chewiness. These results might be explained with the rheological properties observed during the temperature sweep analysis (Fig. 3). In fact, the batters containing DF lentil protein showed the development of the highest G' modulus during heating, meaning the formation of the strongest viscoelastic network (Table 2). Moreover, it should be considered that lentil protein is characterized by a higher content of globular protein compared to mung bean (Shrestha et al., 2022) and chickpea (Grasso et al., 2022), which may be responsible for the strong gelling ability of the protein.

Table 3. Mean value, standard deviation, and results of the statistical analysis two-way ANOVA of the physical characteristics of sponge cakes prepared with the aqueous dispersion of chickpea (C), lentil (L), and mung bean (M) proteins at 20, 30, and 40 %. CTRL: control sponge cake.

Sample	Specific volume (mL g^{-1})	Thickness variation (%)	Firmness (N)	Springiness	Chewiness (N)	Cohesiveness	≤0.5 mm^2	0.5$<$x$<$1 mm^2	≥1 mm^2	n. cells/cm^2
C20	1.84 ± 0.03d	122 ± 2d	4.67 ± 0.27f	0.73 ± 0.01abc	1.01 ± 0.13d	0.30 ± 0.04d	76.61 ± 8.74a	12.31 ± 2.62b*	13.69 ± 3.50d	16.72 ± 2.91ab*
C30	2.12 ± 0.06abc	126 ± 6d	6.58 ± 0.25de*	0.71 ± 0.02abc	1.58 ± 0.03cd	0.34 ± 0.01cd*	58.72 ± 4.26b*	13.31 ± 1.17ab*	25.78 ± 2.19bc*	17.31 ± 0.44ab*
C40	2.21 ± 0.17ab	152 ± 4b*	9.33 ± 0.33c	0.74 ± 0.03abc	2.11 ± 0.15bc*	0.31 ± 0.01d	54.94 ± 1.03b*	13.48 ± 0.10ab*	31.58 ± 1.13ab*	16.00 ± 0.81b*
L20	2.13 ± 0.02abc	138 ± 2c*	6.30 ± 0.10e	0.70 ± 0.02bcd	1.51 ± 0.05cd	0.34 ± 0.02cd*	59.09 ± 4.83b*	17.13 ± 3.06a*	23.79 ± 1.77c*	20.13 ± 0.31a
L30	2.09 ± 0.08abc	154 ± 6b	11.40 ± 0.40b	0.80 ± 0.01ab*	2.93 ± 0.07b*	0.32 ± 0.01cd	51.69 ± 1.69b*	14.77 ± 0.37ab*	33.53 ± 2.06a*	15.22 ± 0.47b*
L40	2.29 ± 0.05a*	170 ± 2a	15.20 ± 0.17a	0.69 ± 0.01cd	5.53 ± 0.02a	0.53 ± 0.01ab	59.34 ± 0.07b*	13.65 ± 0.74ab*	27.01 ± 0.81abc*	17.91 ± 0.97ab*
M20	1.86 ± 0.05d	106 ± 6e	3.45 ± 0.05g	0.61 ± 0.04d	1.03 ± 0.08d	0.42 ± 0.01c*	59.32 ± 4.16b*	12.97 ± 0.20ab*	27.72 ± 3.96abc*	17.59 ± 0.03ab*
M30	1.95 ± 0.05cd	110 ± 2e	7.46 ± 0.08d*	0.48 ± 0.08e	1.54 ± 0.01cd	0.43 ± 0.01bc*	56.24 ± 3.76b*	12.4 ± 2.06ab*	31.36 ± 1.70ab*	16.94 ± 1.81ab*
M40	2.02 ± 0.03bcd	122 ± 2d	9.64 ± 0.02c	0.81 ± 0.02a*	4.73 ± 0.06a	0.63 ± 0.06a	52.84 ± 0.57b*	15.05 ± 1.41ab*	32.11 ± 1.99ab*	14.66 ± 0.91b*
p-value type of legume × amount	0.012	*0.005*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	0.002	0.056	<0.001	0.006
CTRL	2.4 ± 0.09	142 ± 6	7.14 ± 0.36	0.85 ± 0.01	2.48 ± 0.31	0.42 ± 0.01	57.45 ± 2.39	14.14 ± 1.69	28.41 ± 0.70	17.66 ± 2.09
C	2.06 ± 0.20b	133 ± 15b*	6.86 ± 2.04b*	0.73 ± 0.02a*	1.57 ± 0.49c*	0.31 ± 0.03c*	63.42 ± 11.15a*	13.03 ± 1.53b*	23.69 ± 8.19b*	16.68 ± 1.63a*
L	2.17 ± 0.10a	154 ± 14a*	10.97 ± 3.87a*	0.73 ± 0.05a*	3.32 ± 1.77a*	0.40 ± 0.10b*	56.71 ± 4.55b*	15.18 ± 2.21a*	28.11 ± 4.53a*	17.75 ± 2.20a*
M	1.94 ± 0.07c	113 ± 8c	6.85 ± 2.74b*	0.64 ± 0.15b	2.43 ± 1.78b*	0.49 ± 0.11a*	56.13 ± 3.98b*	13.47 ± 1.74ab*	30.39 ± 3.12a*	16.40 ± 1.68a*
p-value type of legume	*<0.001*	*<0.001*	*<0.001*	*0.001*	*<0.001*	*<0.001*	0.002	0.031	<0.001	0.086
20	1.94 ± 0.14c	122 ± 14c*	4.81 ± 1.28c	0.68 ± 0.05b	1.18± 0.40c*	0.35 ± 0.04b*	65.01 ± 10.25a*	14.14 ± 3.03a*	21.73 ± 6.86b*	18.15 ± 2.12a*
30	2.05 ± 0.14b	130 ± 20b*	8.48 ± 2.25b*	0.66 ± 0.15b	2.02 ± 0.71b*	0.36 ± 0.05b*	55.55 ± 4.28b*	13.49 ± 1.59a*	30.23 ± 3.87a*	16.49 ± 1.36b*
40	2.17 ± 0.18a	148 ± 21a*	11.39 ± 2.87a*	0.74 ± 0.06a*	4.12 ± 1.59a	0.49 ± 0.15a	55.71 ± 2.93b*	14.06 ± 1.09a*	30.23 ± 2.71a*	16.19 ± 1.61b*
p-value amount	*<0.001*	*<0.001*	*<0.001*	*0.002*	*<0.001*	*<0.001*	<0.001	0.671	<0.001	0.009

Different letters for the same parameter mean significant differences according to the Tuckey's HSD test (p≤0.05). * means that the value is similar to the control (CTRL) according to the Dunnett's test (p>0.05). n = 4

The variable "amount of protein" also showed a high significant effect on the textural properties, and accordingly, higher DFp concentrations led to a significant increase of firmness and of the other textural indices. Therefore, the products containing 40% of DFp showed the highest firmness, springiness, chewiness and cohesiveness. However, this behavior is in contrast with the increase of the specific volume recorded when higher concentration of DFp were used. In particular, it is generally reported that the firmness of cakes is inversely correlated with the specific volume (Sahagún et al., 2018). In fact, higher protein content in the batter can increase the overall firmness of the product owing to a combination of factors including i) the gelling ability and the formation of a stronger protein network (Yazici and Ozer, 2021); ii) the reduction of the free water due to the water absorption capacity of the vegetable protein (Majzoobi et al., 2014).

The textural indices of all the trials showed some similarities with the CTRL, according to Dunnett's test, except for the C20. In fact, CTRL showed a firmness of 7.14 N, which was similar to the M30 and C30. Overall, these results indicate that different textural properties can be obtained by changing the protein source and the amount of protein, in order to obtain products with the desired texture compared to the egg-based cake.

The image analysis was carried out to evaluate the crumb structure of the sponge cakes, and the results are reported in Table 3. Slight but significant differences were detected in the three porous classes under investigation and in the cell density. In particular, the crumb structure was characterized by the presence of small cells, with the majority of the observations, i.e., 52-59%, having an area ≤0.5 mm^2. The C20 showed the highest porosity in this dimensional range, whereas the other trials showed similar values compared to the CTRL. C20 also showed the lowest percentage of pores with an area ≥ 1mm^2, and therefore this trial showed the major differences compared to the crumb structure of the CTRL. The other trials showed slight but significant differences among them, without following a clear pattern considering the species and the concentrations. The cell density was always similar to the CTRL. The variable "type of legume" was significant for the porous classes and not significant for the cell density. It can be observed that the chickpea-based cakes were characterized by the highest presence of pores with an area ≤0.5 mm^2, and the lowest quantity of pores with an area ≥ 1mm^2 (Yamsaengsung et al., 2010). The protein amount showed a significant effect on the crumb porosity, and the cakes produced with 30% and 40% DFp showed the highest presence of large pores (i.e., with an area ≥ 1mm^2). This confirms that high DFp content is necessary to imitate the egg behavior in sponge cake preparation. Overall, all these trials showed similarities with the CTRL, highlighting that DFp allowed to imitate the egg behavior in cake preparation. This is particularly important, considering that the crumb structure is reported to be worse in the cakes produced with egg replacers compared to the controls and that to overcome this drawback, emulsifiers are often used (Yazici and Ozer, 2021). Moreover, it was previously reported that the increased viscosity of batters containing pea protein hampered the air incorporation during mixing, causing a reduction of the cell dimension (Bustillos et al., 2020; Hedayati et al., 2022; Crawford et al., 2023). Therefore, it has been suggested that a vigorous mixing is then necessary to overcome the drawbacks given by the protein addition (Bustillos et al., 2020). The crumb cell dimensions are generally used as a quality parameter for bakery goods (Yazici and Ozer, 2021) and they contribute to the textural properties of the products (Yazici and Ozer, 2021; Hager et al., 2013). However, it should be reminded that alongside the cell dimension, the surrounding matrix, i.e., the cell wall, also contributes to the mechanical strength of the product (Hager et al., 2013). In fact, no significant correlations were found between the results of the image analysis and the textural evaluation of the sponge cakes (data not shown). Therefore, it is reasonable to assume that the viscoelastic properties of the batters may better explain the phenomena occurring during baking, which are responsible for the quality of the product.

Chemical determinations

The chemical characteristics of the sponge cakes are presented in Fig. 4. Protein and lipid contents proportionally increased as the concentrations of DFp increased in the formulation. The protein content of the formulations containing 30% of DFp was similar to the CTRL, and it was significantly higher in the trials containing 40% of DFp. However, egg is considered as a balanced source of protein in terms of amino acid composition (Réhault-Godbert et al., 2019), whereas legumes and cereals themselves are not able to provide a balanced amino acid intake (Lu et al., 2023). By contrast, the combination of them is a good strategy to balance the amino acid composition (Jarpa-Parra et al., 2017). Further investigation on nutritional value and protein digestibility can be the object of future studies.

The lipid content of the CTRL was higher than all the formulations containing the egg replacer, with the only exception of C40. In fact, the whole egg usually has a lipid content of 9.5 g/100g (Réhault-Godbert et al., 2019), which is higher than the lipid content of the legume proteins used in this study.

Phytic acid was also determined because it is present in legume flours and has been observed to follow the protein fraction during the dry fractionation process (De Angelis et al., 2021). Indeed, it proportionally increased as higher concentrations of DFp were used in the cake formulations. The CTRL showed a phytic acid content of 0.02 g/100 g which was only similar to the C20 formulation. Wheat flour also contains phytic acid in a range of 0.34 -0.76 g/100g (Feizollahi et al., 2021). Phytic acid is considered an antinutritional factor because it reduces the bioavailability of minerals and micronutrients (Kumar et al., 2021). However, some antioxidant and beneficial health roles have been also associated with phytic acid (Kumar et al., 2021), suggesting that further studies are needed to comprehensively evaluate the nutritional value of the food formulated with this kind of plant-based ingredient.

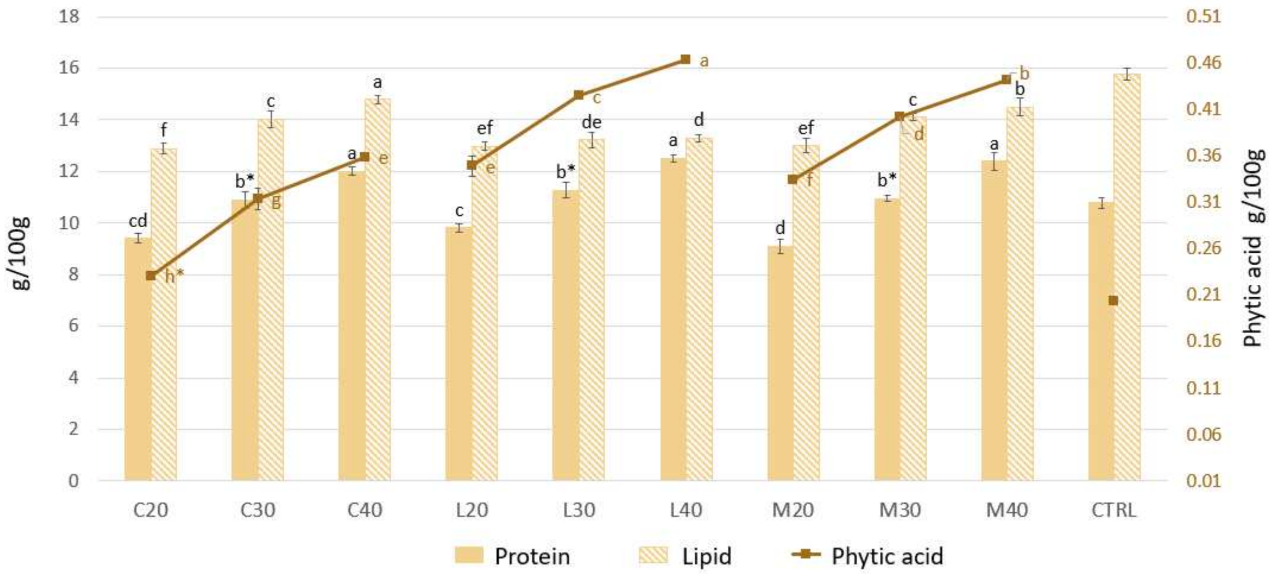

Figure 4. Protein, lipid and phytic acid contents of the sponge cakes prepared with the aqueous dispersion of chickpea (C), lentil (L), and mung bean (M) proteins at 20, 30, and 40 %. * the value is similar to the control (CTRL) according to Dunnett's test (*p*>0.05) *n* = 4.

Sensory analysis

The heatmap showed in Fig. 5 describes the results of quantitative descriptive sensory analysis (QDA) carried out to evaluate the sponge cakes in terms of crust and crumb color, sweetness, legume flavor and astringency. The differences were particularly evident for the crust color, which was evaluated darker in the cakes produced with the egg replacer compared to the CTRL, regardless of the species and of the concentration. The darkening effect of the protein incorporation in bakery goods was previously reported, and it can be related to an increase of the precursors of the Maillard reaction (Majzoobi et al., 2014; Pico et al., 2019). More heterogeneous values were obtained for the crumb color. In fact, it is mainly affected by the color of the protein ingredient and not by the Maillard reactions or sugar caramelization (Majzoobi et al., 2014). No significant similarities with the CTRL were recorded.

The CTRL cake showed the highest score for sweetness, whereas the addition of egg replacer lowered the sweetness perception, which was always evaluated with a score below 4.5 out of 9. Moreover, the cakes produced with 30 and 40% of DFp showed the lowest sweetness. These results were probably due to the bitter taste reported in other studies for DFp (Chigwedere et al., 2022), which might have lowered the sweetness perception (Wang et al., 2019).

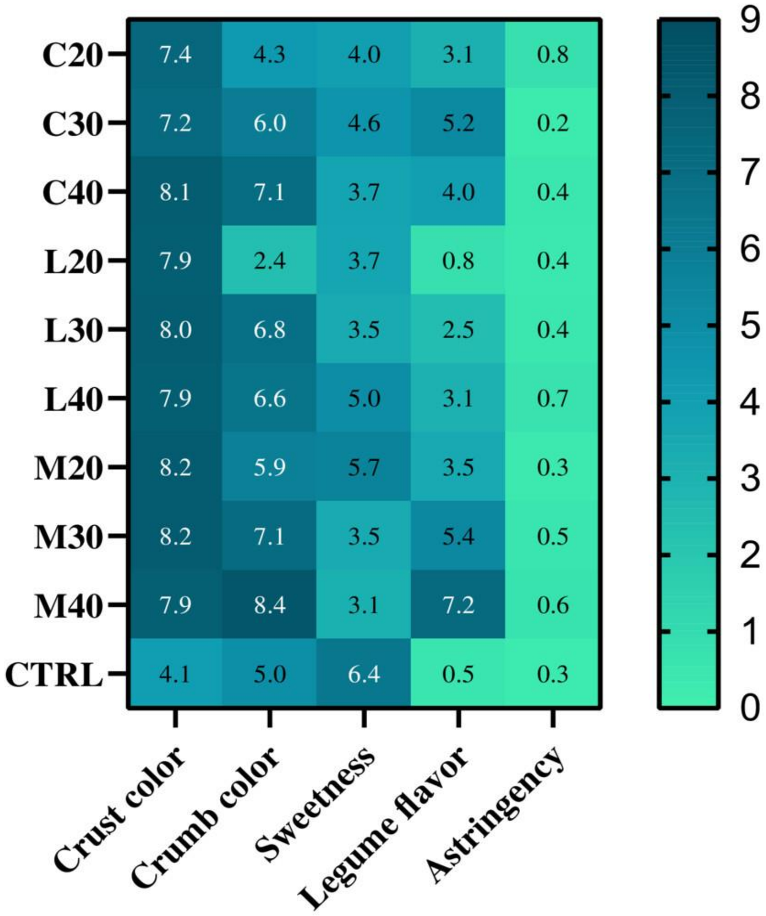

Figure 5. Heat map describing the sensory attributes of the sponge cakes prepared with the aqueous dispersion of chickpea (C), lentil (L), and mung bean (M) proteins at 20, 30, and 40 % evaluated by the quantitative descriptive analysis. CTRL: control sponge cake. *n* = 4.

The legume flavor showed the highest intensity in the cakes formulated with the mung bean protein as egg replacer, and it was affected by the concentration used. By contrast, in the other trials, the legume flavor was slightly perceived, with scores below 4 out of 9. Overall, the legume flavor is one of the critical aspects to consider in new food development, because it is generally not well accepted by consumers (Chigwedere et al., 2022). Therefore, the use of chickpea and/or lentil DFp should be preferred for this kind of formulation, having a lower impact on the sensory properties compared to mung bean. However, further consumer tests could be carried out in order to establish the consumer preference and acceptance for such products.

Finally, the astringency, which is reported to be associated with legumes (Chigwedere et al., 2022), was little perceived by panelists, indicating a low impact of the egg replacers on this sensory descriptor.

Conclusions

The egg replacement is gaining interest worldwide, and the results of this study suggest that dry-fractionated proteins are a sustainable alternative that could be used in sponge cake preparation as egg replacer. However, their introduction in food must be evaluated in order to understand their effect on the physico-chemical quality of the products. The DFp addition resulted in a more pronounced elastic-like behavior of the batters, as seen by the higher G' modulus and the higher apparent viscosity compared to the CTRL formulation. Moreover, the DFp concentration significantly affected the rheological behavior during baking, and lentil-based batters showed the highest G' after the heating treatment. Through the temperature sweep analysis, it was possible to explain the phenomena occurring during baking, which are responsible for the quality features of the products. In fact, higher specific volume, thickness variation, and firmness were found in the products containing the highest concentration of DFp.

All the cakes prepared with DFp showed similarities in the crumb structure compared to the CTRL, regardless of the protein type and concentration. The protein content of the cakes containing at least 30% DFp was similar to the CTRL. However, the addition of DFp was directly related to an increase of the phytic acid content. The sensory analysis highlighted a lower impact of the chickpea and lentil protein on the legume flavor, compared to the mung bean.

The overall results of this study confirm that the eggs in cakes formulation can be substituted by legume protein produced by dry fractionation, which were able to imitate the egg behavior and the physical structure of the products. Furthermore, different textural properties can be obtained by changing the protein source and the amount of protein, in order to obtain products with the desired texture compared to the egg-based cake.

This is particularly important considering that, although the dry fractionation process was comprehensively studied, limited food applications are reported in scientific literature. However, further studies would be needed to evaluate the nutritional characteristics e.g., amino acid composition, and digestibility, of the DFp-based cakes in comparison with the traditional egg-based products, as well as assessing the overall acceptability of the products by carrying consumer acceptability tests.

AUTHOR CONTRIBUTIONS

Davide De Angelis: Conceptualization, Investigation, Formal analysis, Writing - Original draft preparation, Writing - Review & Editing; Vittoria Latrofa: Conceptualization, Investigation, Formal analysis Writing - Original draft preparation; Giacomo Squeo: Conceptualization, Investigation, Formal analysis, Writing - Review & Editing; Antonella Pasqualone: Project administration, Resources, Writing - Review & Editing; Carmine Summo: Conceptualization, Resources, Project administration, Writing - Review & Editing.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

DECLARATION OF INTEREST

The authors have no conflict of interest to declare.

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Chapter 4. Developing a clean labelled snack bar rich in protein and fiber with dry-fractionated defatted durum wheat cake

This chapter is published as:

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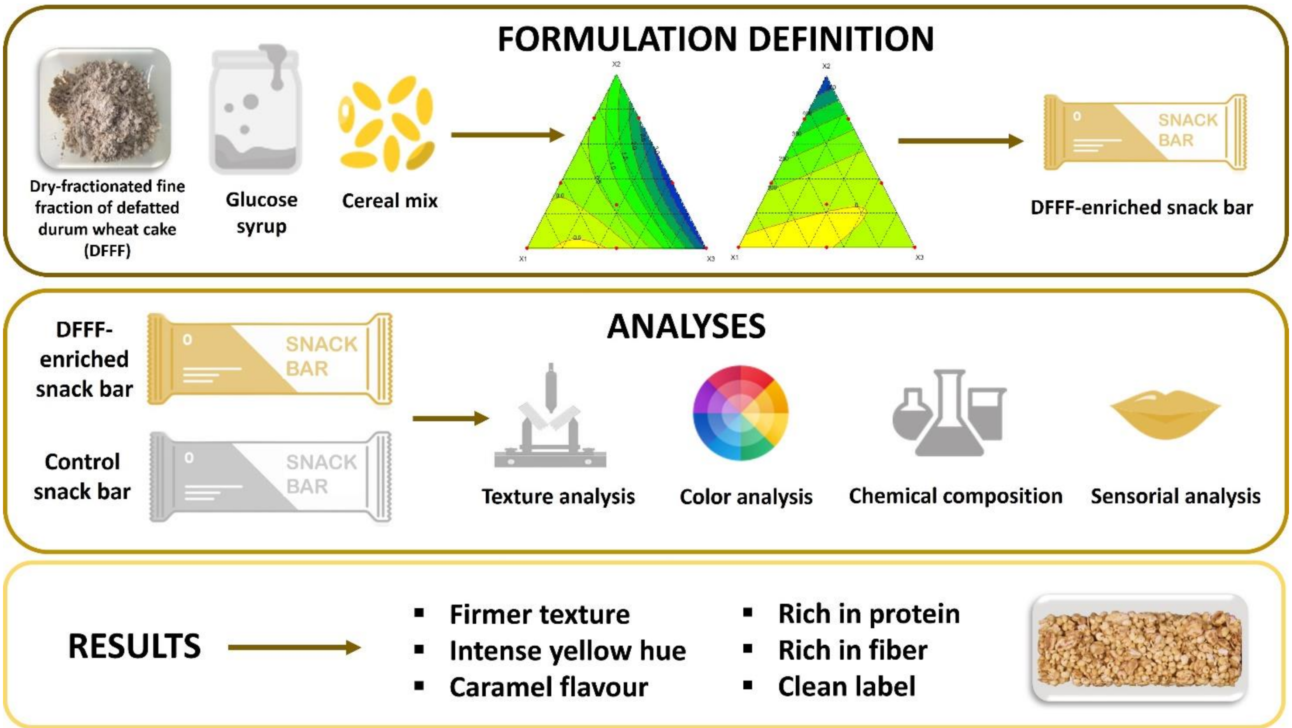
Graphical abstract



Abstract

The shift towards a vegetarian, vegan, or flexitarian diet has increased the demand for vegetable-proteins and plant-based foods. The defatted cake generated during the extraction of lipids from durum wheat (*Triticum turgidum* L. var. *durum*) milling by-products is a protein and fiber-containing waste which could be upcycled as food ingredient. This study aimed at exploiting the dry-fractionated fine fraction of defatted durum wheat cake (DFFF) to formulate a vegan, clean labelled, cereal-based snack bar. The design of experiments (DoE) for mixtures was applied to formulate a final product with optimal textural and sensorial properties, which contained 10% DFFF, 30% glucose syrup, and 60% mix of puffed/rolled cereals. The DFFF-enriched snack bar was harder compared to control without DFFF (cutting stress = 1.2 and 0.52 N/mm^2 and fracture stress = 12.9 and 9.8 N/mm^2 in the DFFF-enriched and control snack bar, respectively), due to a densifying effect of DFFF, and showed a more intense yellow hue due to the yellow-brownish color of DFFF. Another difference was in the caramel flavor, more intense in the DFFF-enriched snack bar. The nutritional claims “low fat” and “source of fiber” were applicable to the DFFF-enriched snack bar according to the EC Reg. 1924/06.

Keywords: Cereal based; texture; upcycling; ready to eat food; design of experiments; texture analysis; nutritional composition.

Introduction

One third of food produced for human consumption is lost or wasted worldwide, accounting for about 1.3 billion tons annually (Fao, 2011) Alongside this, food supply chains generate a large amount of valuable by-products that still could be exploited and valorized for human nutrition. As for the supply chain of durum wheat (*Triticum turgidum* L. var. *durum*), during the milling process a by-product is generated, composed by germ, bran and debranning fractions (Onipe et al., 2021). Being rich in high biological value proteins, minerals, fibers and lipids, this by-product can be further exploited (Prueckler et al., 2014; Onipe et al., 2015). Indeed, the extraction of the lipid fraction, rich in vitamins and essential fatty acids, has been proposed (Squeo et al., 2022), and durum wheat oil has been used for the preparation of biscuits and focaccia (Vurro et al., 2022a; Vurro et al., 2022b). The lipid extraction, however, generates a second by-product, namely the defatted durum wheat cake. To upcycle the defatted cake, after stripping and micronization, the dry-fractionation process can be applied, allowing to obtain a coarse fraction rich in starch and fiber, and a fine fraction rich in proteins (Schutyser et al., 2011). The coarse fraction of dry-fractionated cereal flours has been already proposed as ingredient in different food products, such as meat analogues and spaghetti (Bühler et al., 2022; Verardo et al., 2011). In addition, also the fine fraction can be used to fortify foods (Silventoinen et al., 2021), with the advantage of being derived by a more sustainable method of protein enrichment compared to wet concentration, which uses an alkaline extraction followed by isoelectric precipitation (Schutyser et al., 2011).

The sustainability of food systems is a hot topic increasingly considered by researchers, national/international organizations, and consumers. Also, the recovery of proteins from vegetable waste and by-products matches the general request of alternative plant proteins to replace the animal ones (Pojić et al., 2018). In fact, the shift towards a vegetarian, vegan, or flexitarian diet has increased the demand for vegetable-proteins and plant-based foods (Alcorta et al., 2021; GFI, 2021). The reduction of animal proteins is associated with well-being and health, both being drivers that play a pivotal role in influencing the food choices of many consumers, who integrate nutrition with sport activities (López-Martínez et al., 2022). To support this healthy lifestyle, consumers increasingly choose foods rich in proteins and fibers, usually marketed in the form of snack bars (Westberg et al., 2022; Arribas et al., 2019). In addition, the fast pace of life further concurs to the “snacking” effect, mostly based on the consumption of snack bars.

Being sweet snacks considered unhealthy for the presence of high levels of sugars, food companies are releasing snacks formulated in a healthier way, incorporating protein isolates to increase protein content (Gill and Meena, 2020) and preferring clean labelled formulations (Ciurzyńska et al., 2019; Anandito et al., 2021). Though specific regulations/legislations of clean label do not exist, and a clear definition has not been established (Asioli et al., 2017), this new trend in food products, mostly referring to the absence of additives, has been taken up by a multitude of food industry stakeholders (Osborn et al., 2015). Indeed, many additives can be included in the formulation of snack bars. Among the most used, sorbitol and polyols, responsible of a laxative effect, are sugar replacers, while lecithin is the most common emulsifier, and glycerol is added as gelatinizing agent, followed by coloring agents and artificial flavors to improve the sensory properties (Boukid et al., 2022). Moreover, saturated fats, involved in cholesterol increase and cardiovascular disease, are reported among the variety of ingredients commonly used in the snack bars (Boukid et al., 2022).

Over the past few years, several studies have concerned the re-use of food industry by-products (such as bran of black rice and corn, banana peel powder, sunflower meal, and jackfruit seeds flour) in the formulation of snack bars (Anandito et al., 2021; Singh et al., 2022; Baurina et al., 2021; De Sousa et al., 2019; Meethal et al., 2017). These studies have confirmed the increasing interest towards this food category, highlighting its suitability as a recipient for fortification and functionalization. However, the re-use of defatted durum wheat cake, which can be easily dry-fractionated to obtain a protein-rich flour, has not been considered so far.

In this framework, this study aimed at exploiting the dry-fractionated fine fraction of defatted durum wheat cake (DFFF) to formulate a vegan, clean labelled, cereal-based snack bar, evaluating the influence of the ingredients on the textural, sensory, and nutritional properties of the final product.

Materials and methods

Basic Ingredients

DFFF was provided by Casillo Next Gen Food srl (Corato, Italy). Its preparation was as follows. The durum wheat milling by-product was composed of a mix of germ, bran and debranning fractions and it was submitted to the extraction of the oily fraction using *n*-hexane, as described in the study by Squeo et al. (2022). The remaining cake was micronized using an impact mill (UPZ 100, Hosokawa-Alpine, Augusta, Germany). The mill speed was set at 15000 rpm and the feed rate at 3 kg/h. Then, an air classifier (ATP 50, Hosokawa-Alpine, Augusta, Germany) was used to dry-fractionate the micronized cake flour into coarse and fine fractions. The speed of the grader wheel was set at 3000 rpm and the air flow rate at 50 m^3/h. The fine fraction was recovered, having a *n*-hexane residual content <5 ppm, i.e. food grade. The chemical composition of DFFF is shown in the Figure 1.

Puffed hulled millet (Vivibio, Villareggia, Italy), 5-grain rolled cereals (20% oat, 20% barley, 20% rice, 20% rye, 20% wheat) (Fiorentini, Troffarello, Italy) and puffed spelt (Selex, Trezzano, Italy) were purchased from a local retailer.

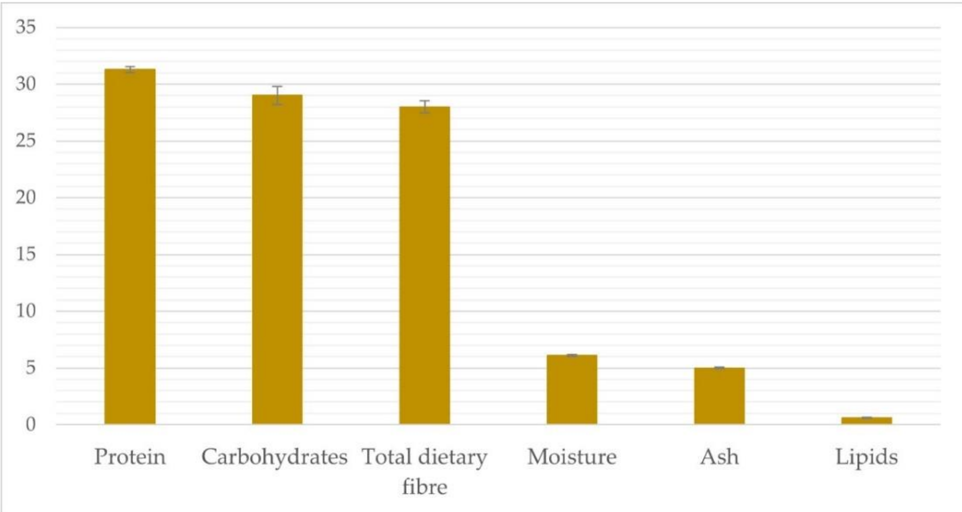


Figure 1. Proximate composition (g/ 100 g) of dry-fractionated fine fraction of defatted durum wheat cake (DFFF).

Experimental Design and Preparation of the Snack Bars

The design of experiments (DoE) for mixtures (Squeo et al., 2021) was applied to formulate a product with optimal textural and sensorial properties. Three factors were considered, that were the three main ingredients, namely DFFF, glucose syrup, and a cereal mix composed of puffed hulled millet, 5-grain rolled cereals and puffed spelt (35:40:25 w/w/w). The quantitative ranges were 10-50%, 30-60%, and 20-60% for DFFF, glucose syrup, and cereal mix, respectively. Then, from all the possible mixtures in the defined experimental domain, 8 unique formulations were chosen (Table 1) by a D-optimality criterion (Leardi et al., 2006), to model a special cubic response.

To prepare the snack bars, glucose syrup (Ambrosio, Striano, Italy) and DFFF were manually mixed and heated in a pan on an induction heater (G3 Ferrari, Rimini, Italy) at the power of 2000 W, until the complete formation of a cream was reached (about 5 min). Then, the cereal mix was added and manually mixed to distribute it homogeneously into the cream, followed by pouring in a silicone mold (FantasyDay, Changan Town, China) having six 2-cm thick cavities of 11.5 x 3.8 cm, and cooking in a ventilated oven (Smeg, Guastella, Italia) for 5 min at 120 °C. The snack bars were allowed to cool down at room temperature (20 ± 1 °C), then were extracted from the molds.

Three snack bars for each formulation of the experimental design were prepared (*n* = 3). A control snack bar, without DFFF, was prepared with 33.3 g/100 g of glucose syrup and 66.6 g/100 g of cereal mix.

Table 1. List of the experiments performed.

Experiment	Dry-fractionated fine fraction of defatted durum wheat cake – X1 (g/100 g)	Glucose syrup – X2 (g/ 100 g)	Puffed/rolled cereal mix – X3 (g/ 100 g)
1	50	30	20
2	20	60	20
3	10	60	30
4	10	30	60
5	10	45	45
6	35	45	20
7	25	40	35
8	30	30	40

Determination of the Chemical Composition

Protein content (N x 5.7) was determined according to the method AOAC 979.09 (AOAC, 2006). The lipid content was determined through the Soxhlet apparatus according to the method AOAC 945.38F (AOAC, 2006), using diethyl ether as extraction solvent. The total dietary fiber was analysed by the enzymatic-gravimetric procedure as described in the method AOAC 985.29 (AOAC, 2006). Moisture and ash content were determined according to the AOAC methods 925.10 and 923.03 (AOAC, 2006), respectively. The available carbohydrate content was determined by difference, subtracting the content of protein, lipid, fiber, moisture and ash to 100. Energy value was calculated by using the Atwater general conversion factors. All determinations were carried out in triplicate.

Texture Analysis

The textural properties of the snack bars were determined by a cutting test and a three-point bending test. A texture analyzer, equipped with a 1 kN load cell (Z1.0 TN, Zwick Roell, Ulm, Germany) was used for both the tests.

The cutting test was carried out as described in Costantini et al. (2021) with some modification. The sample (11.5 × 3.8 × 2 cm, L × W × H) was positioned on the base in order to be cut by the 50 mm length blade on its short side. positioned 25 mm from the surface of the sample, The blade descended at a speed of 1 mm/s, until the sample was cut. The cutting stress, i.e. the maximum force applied on the surface until the sample was completely cut (N/mm^2), and the deformation until the cut (mm) was measured.

The three-point bending was carried out as reported in Pasqualone et al. (2019) with a few modifications. The texture analyzer was equipped with a 1 kN load cell, a metal breaking probe and two metal supports, 6 cm apart from each other, to hold the whole sample (11.5 × 3.8 × 2 cm, L × W × H). The sample was placed on the two supports, and the probe (positioned 25 mm above the sample) descended on it at a speed of 5 mm/s. The fracture stress (N/mm^2) i.e., the maximum force required to break the sample, and the deformation until rupture (mm) were measured.

Data acquisition was accomplished through the TestXPertII v3.41 software (Zwick Roell, Ulm, Germany). The analysis was carried out in triplicate.

Sensory Analysis

The sensory features were evaluated by a panel consisting of 8 trained members, aged between 26 and 56 years. The panelists did not suffer from any food intolerances and allergies, received information on the objectives of the study, and provided written informed consent.

At first, the snack bars corresponding to the 8 different formulations (Table 1) were evaluated on a 0-9 score range (0 = minimum; 9 = maximum) for their stickiness (a critical parameter for these products) and acceptability. The results of this evaluation, together with the textural data, allowed to select the optimal snack bar. Then, both the optimal and the control snack bar were submitted to Quantitative Descriptive Analysis (QDA) for detailed sensory profiling. Eight sensory descriptors were evaluated on a 0-9 score range (0 = minimum; 9 = maximum intensity), regarding visual appearance (homogeneity of the distribution of cereals, brightness, yellowish color), flavor (cereal-like, toasted, caramel and abnormal), and taste (sweet).

Color Analysis

The snack bars had a highly heterogeneous color that could not be analysed directly with a colorimeter and, for this reason, an image analysis and color elaboration was carried out. An RGB image was collected through a Canon EOS 600D camera equipped a Canon EF-S 18-200mm f/3.5-5.6 IS lens (Canon Inc., Tokyo, Japan), at a 50-cm distance between the lens and the sample. The optimal and control snack bars, placed inside a white plastic box sized 49 x 51 x 50 cm having a black base, were photographed from above. The image (2193 × 2683 pixels) was imported into MATLAB R2021a (The MathWorks Inc., Natick, MA, USA) and processed as follows. After masking and background removal, done by using the HYPER-Tools toolbox (freely available at www.hypertools.org) (Amigo et al., 2015), the image was converted from RGB color space to *L*a*b** color space, thus obtaining the respective values of brightness, red and yellow indices per each pixel. Then, the absolute frequency distribution and the cumulative frequency distribution of the pixels were calculated per each *L*a*b** index to highlight differences in the color characteristics of the snack bars.

Statistical Analysis

The mixture experimental design was set up and analysed using CAT (Chemometric Agile Tool) software (Leardi et al., 2013), freely downloadable from http://gruppochemiometria.it/index.php/software). All data were expressed as the mean ± standard deviation (n = 3). One-way analysis of variance (ANOVA) was performed, followed by the Tukey test for multiple comparisons, using Minitab 17 (Minitab Inc., State College, PA, USA).

Results and discussion

Identification of the Optimal Formulation

Figure 2 depicts the contour plots of the cutting stress (A), the fracture stress (B), the sensory acceptability (C) and the stickiness (D) of the experimental snack bars in the defined domain. DoE allowed to reach a comprehensive understanding of the ingredients' effect on the snack bar properties, and to select the better formulation. A snack bar, indeed, is a mixture of ingredients which substantially retain their original physical characteristics, generally very different from each other, so their relative quantities greatly influence the features of the final product.

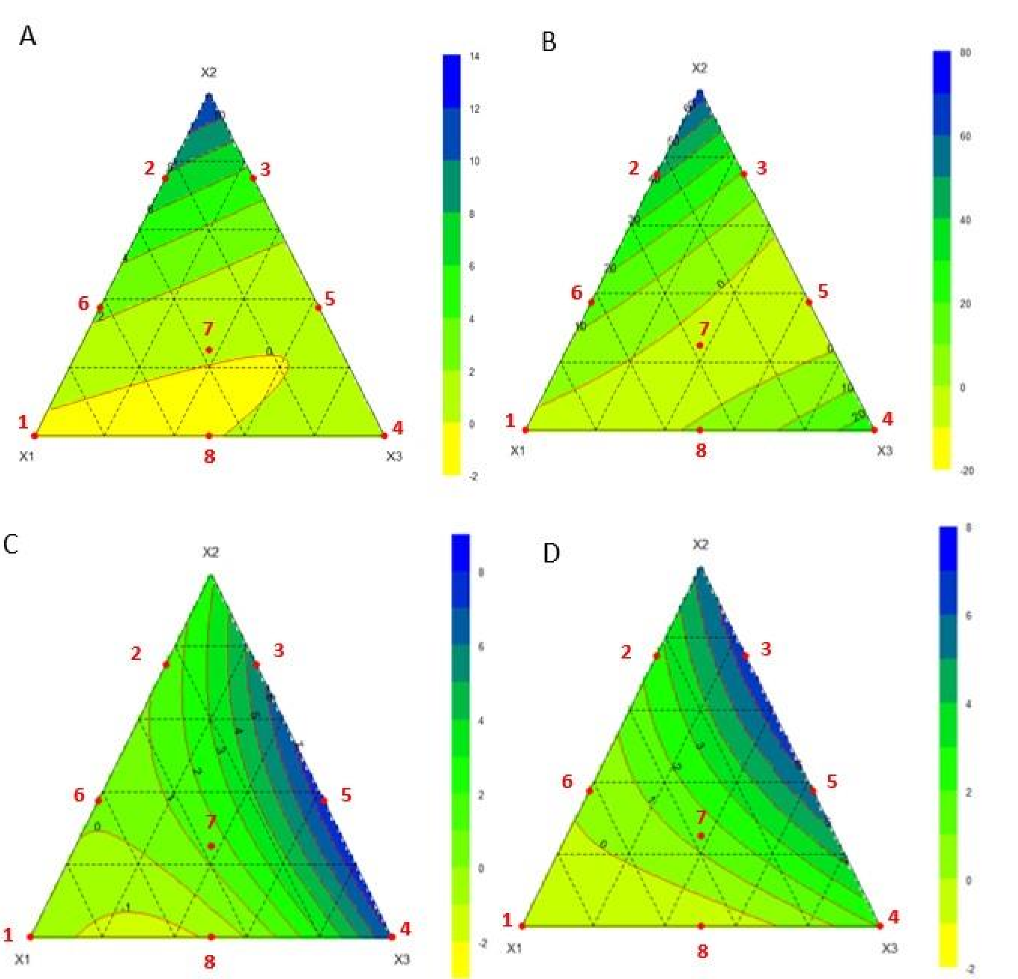


Figure 2. Contour plots of the cutting stress (N/mm^2, A), fracture stress (N/mm^2, B), sensory acceptability (C), and stickiness (D) of the experimental snack bars. X1 = dry-fractionated fine fraction of defatted durum wheat cake (DFFF); X2 = glucose syrup; X3 = puffed/rolled cereal mix. Red point represents the experiments carried out; numbers 1-8 correspond to the formulations reported in Table 1.

The cutting and fracture stress, determined by measuring the maximum force required to cut or break the sample, respectively (Figs. 2A and 2B), were considered because they are known to be related to sensory firmness and stickiness to the teeth (Kim et al., 2009), which are important characteristics for the snack bars. The fitting of the responses, however, was not excellent, showing in both the cases an R^2 equal to 0.6. This was likely due to the high physical inhomogeneity of this kind of product, which brought to a great variability in the responses, making the modelling more difficult. Nonetheless, from the contour plots useful information about the effect of the ingredients on the mechanical features of the snack bar could be caught. Indeed, the cutting and fracture stress values, and thus the hardness of the snack bars, progressively increased by rising the proportion of X2 (glucose syrup). Therefore, the highest amount of glucose syrup gave rise to very hard bars, as observed close to the X2 vertex. On the other hand, the experimental region close to the X1 vertex corresponded to the worst results, due to a total lack of consistency. The snack bars in this region (trials 1 and 8) were impossible to shape properly, showing a powdery and poor structure (Fig. 3).

Figure 3. Experimental snack bars formulated with dry-fractionated fine fraction of defatted durum wheat cake (DFFF), glucose syrup and puffed/rolled cereal mix. Numbers 1-8 correspond to the formulations reported in Table 1.

The experiments 1 (50% DFFF, 30% glucose syrup, 20% cereals) and 8 (30% DFFF, 30% glucose syrup, 40% cereals) highlighted that the snack bar could not be formed when DFFF was ≥ glucose syrup. Indeed, DFFF was rich in protein and fiber, so its increase would be nutritionally valuable (Ciudad-Mulero et al., 2019), but being a powdery ingredient, an excessive amount disrupted the structure of the snack bar, especially when the amount of glucose syrup was too low. At the same time, high amounts of glucose syrup excessively increased the hardness. By increasing the amount of the cereal mix (X3), a moderate and positive effect on the structure was observed. Therefore, for the textural features, the best experiments were those in the centre of the domain (point 7), at the X3 vertex (point 4), and along the edge X3-X2 (points 3 and 5) (Figs. 2A and 2B). Together with the textural properties, other two parameters were chosen for the selection of the optimal formulation, i.e. sensory acceptability, which is fundamental for food products in general, and stickiness, which is important especially for the snack bars. The responses for these two parameters are depicted in figures 2C, and 2D, respectively. In this case the fitting of the responses was better than the textural parameters, showing an R^2 equal to 0.9 for the sensory acceptability and 0.7 for the stickiness, both markedly influenced by variations in the ingredient ratio. The stickiness was very high in the upper part of the edge X3-X2 (experiments 3 and 5). The highest sensory acceptability and the lowest stickiness were observed in the experimental region close to the X3 vertex (point 4).

In the light of these results, the most suitable trade-off was considered the formulation corresponding to the X3 vertex (experiment 4), i.e., 10% DFFF, 30% glucose syrup, and 60% cereals. This well-structured bar, neither too sticky, nor too hard, was the optimal one and was then further characterized for its nutritional, textural and sensorial features.

Nutritional, Textural and Sensorial Characterization

Table 2 shows the results of the nutritional composition of the DFFF-enriched snack bar, compared with the control snack bar, prepared without DFFF. The addition of DFFF caused a significant reduction of the moisture and an increase in protein content respect to the control. These results were expected, being DFFF an ingredient high in protein and low in moisture. In particular, the increase in proteins highlighted the effectiveness of using a dry-fractionated ingredient, considering that studies involving the addition of other cereal by-products, such as black rice bran and maize bran (Anandito et al., 2021; De Sousa et al., 2019; Garcia et al., 2012), did not achieve a significant increase in protein content. One DFFF-enriched snack bar (weight = 33 g) covered 6.4% of the daily reference intake for proteins, while the control snack bar provided only 4.3% of the reference intake. The consumption of plant-based proteins has a positive effect on health, since it minimizes the cardiometabolic risk factors and is inversely correlated to hypertension, obesity, and insulin resistance (Zhubi-Bakija et al., 2021). DFFF is also rich in fiber (Fig. 1). Therefore, another positive result of the incorporation of DFFF was the enrichment in total dietary fiber (Table 2), recommended to improve the metabolism of gut microbiota and to reduce the risks connected with diabetes, obesity, and dyslipidaemia (Camerotto et al., 2019). Moreover, the reached amount of fiber makes possible labelling the DFFF-enriched snack bar as "source of fiber" according to the current rules (Regulation (EC) No 1924/2006), as it meets the minimum requirement of 3 g of fibers per 100 g of product. This amount was higher than the level reached by adding other by-products of the food industry, such as pumpkin seed flour or carrot powder (Umme et al., 2021; Mathu et al., 2022). In detail, a single DFFF-enriched snack bar (33 g) provided 4.2% of the daily intake recommended by the EFSA for dietary fibers (i.e., 25 g) (EFSA, 2010), while the control bar provided 3.0% of the reference intake.

The lipid content did not show a significant difference between the two snack bars. The observed amount makes possible labelling them as “low fat” according to the EC Reg. 1924/2006 (Regulation (EC) No 1924/2006) (no more than 3 g of fat in 100 g). The observed lipid content was considerably lower than in snack bars formulated with other by-products, such as tilapia frames or roasted rice bran (Garcia et al., 2012; Zulaikha et al., 2022).

The carbohydrates remained the main constituent of the snack bars, but they were significantly lower in the DFFF-enriched bar, than in the control one.

The two types of snack bars showed a very similar energy value, providing 6% of the daily reference value of 2000 kcal (European Parliament, 2011).

Table 2. Nutritional composition of the experimental snack bars. DFFF = dry-fractionated fine fraction of defatted durum wheat cake.

Parameter	DFFF-enriched snack bar			Control snack bar		
	Amount for 100 g	Amount per unit (33 g)	% Daily value per unit (33 g) *	Amount for 100 g	Amount per unit (33 g)	% Daily value per unit (33 g) *
Moisture (g)	8.3±0.11[b]	2.7	-	9.3±0.17[a]	3.2	-
Proteins (g)	9.7±0.24[a]	3.2	6.4	6.5±0.08[b]	2.3	4.3
Total dietary fiber (g)	3.2±0.20[a]	1.1	4.2	2.3±0.31[b]	0.8	3.0
Lipids (g)	1.4±0.09[a]	0.5	0.7	1.4±0.04[a]	0.5	0.7
Carbohydrates (g)	76.4±0.36[b]	25.2	9.8	80.1±0.63[a]	26.4	10.2
Energy value (kcal)	363	120	6.0	364	120	6.0

*Energy, proteins, lipids, and carbohydrates were calculated according to the daily reference intakes reported in the Annex XIII of the EU Reg. 1169/2011 (European Parliament, 2011); total dietary fiber was calculated according to the daily reference intake reported in the EFSA recommendation (EFSA, 2010). Different letters indicate significant differences at $p < 0.05$.

As for the texture analysis of the snack bars, both cutting and bending tests, which had helped also in selecting the optimal formulation, highlighted significant textural differences between the DFFF-enriched snack bar and the control, with the former harder than the latter. Results are shown in Table 3. The observed increase in cutting and fracture stress was likely due to the densifying effect of DFFF when added to glucose syrup, leading to a thick cream that made the snack bar become very compact and hard. These results were correlated with the higher extent of deformation observed in the DFFF-enriched bar respect to the control. This indicated a greater effort needed to chew the DFFF-enriched snack bar compared to the control and, more in general, evidenced the ability of the new formulation to tolerate higher stress. Foods that need a longer chewing could reduce the appetite and then, also the energy intake (Miquel-Kergoat et al., 2015). Therefore, a harder texture is desirable from the nutritional point of view. Zulaikha et al. (2021) and Silva et al. (2018) observed a similar increase in hardness following the addition of powdery ingredients, such as tilapia frames and marolo pulp flour, to snack bars.

As for the color of the snack bars, a high heterogeneity was found along their surface, as expected in this kind of products (Fig. 3). As a consequence, a direct colorimeter evaluation was not sufficiently reliable, justifying the need of imaging approaches to better evaluate the product's color. Figure 4A shows the image of the snack bars reconstructed according to the $L^*a^*b^*$ values of each pixel. In both the snack bars, the surface of the cereal grains showed higher values of brightness (L^*) that could be explained by the shiny coating formed by the glucose syrup. The pixel distribution for the L^* value (Figure 4B) was bimodal, i.e. presented two main peaks, indicating strong contrast within the surface brightness in different parts of the snack bar, due to the heterogeneity of the assembled ingredients. The curves of the cumulative distribution showed that the control bar had a higher percentage of pixels with high L^* values respect to the DFFF-enriched snack bar, supporting the empirical observation (Fig. 4A) that the former was, overall, brighter than the latter.

Table 3. Texture analysis of the experimental snack bars. DFFF = dry-fractionated fine fraction of defatted durum wheat cake.

Parameter	Type of snack bar	
	DFFF-enriched	Control
Cutting test		
Cutting stress (N/mm²)	1.2±0.1[a]	0.52±0.2[b]
Deformation (mm)	16.3±0.29[a]	14.7±0.56[b]
Three point-bending test		
Fracture stress (N/mm²)	12.9±3.9[a]	9.8±4.09[b]
Deformation (mm)	3.6±0.8[a]	2.5±1.07[b]

Different letters indicate significant differences at $p < 0.05$.

A high rate of negative *a** values were recorded in both the snack bars (Fig. 4A), indicating a general trend toward the greenish color, with some surface portions characterized by positive values and thus reddish features. The pixel distribution (Fig. 4B) was very similar between the two snack bars, showing minor differences in the whole red-green feature, but with a higher proportion of reddish pixels in the DFFF-enriched snack bar respect to the control.

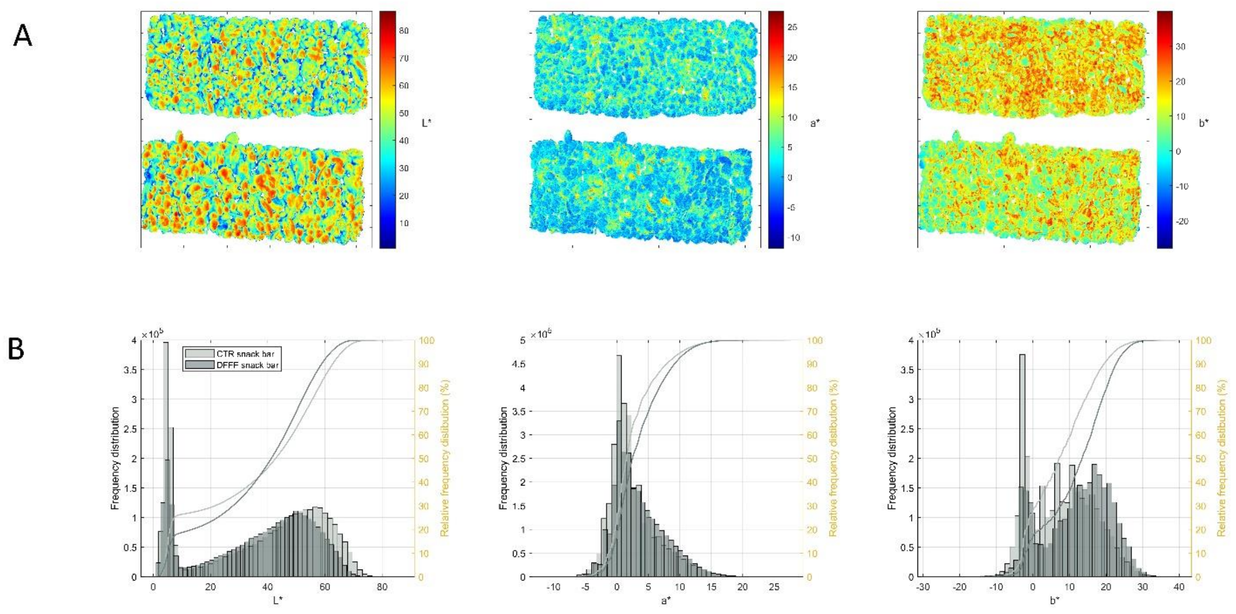


Figure 4. A) *L*a*b** reconstructed images of the DFFF-enriched snack bar (snack bar on the top of each image) and the control snack bar (snack bar on the bottom of each image). B) Pixel absolute frequency distribution (bars) and cumulative frequency distribution (lines) of the control snack bar (light grey) and of the DFFF-enriched snack bar (dark grey) for *L**, *a** and *b**.

A remarkable difference was observed for the *b** index, with the DFFF-enriched snack bar that clearly presented a more intense yellow hue (i.e., positive values) respect to the control bar (Fig. 4A). The DFFF ingredient, indeed, is characterized by a yellow-brownish color that is reflected in a more yellow bar. This empirical observation is supported by the frequency distribution (Fig. 4B) that clearly shows a higher proportion of pixels having high *b** values in the DFFF-enriched snack bar respect to the control. Overall, it could be concluded that the DFFF-enriched snack bar was slightly less bright but redder and yellower than the control bar.

The QDA results are reported in Table 4. The visual evaluation revealed statistically significant differences in the color, with the DFFF-enriched snack bar perceived as more yellow than the control, in agreement with the instrumental analysis. Brightness was also in line with the instrumental results, because the DFFF-enriched bar was significantly less bright than the control, probably because the addition of DFFF diminished the glossy appearance of the surface. From a practical point of view, the corroboration of the results of the instrumental color evaluation and of the sensory evaluation suggest that an image processing technology may be implemented on the production process for the quality control procedures, considering also that the color determination with the classical colorimeters is challenging for such heterogeneous products, and the sensory evaluation requires trained panellists, and it is time consuming. On the contrary, no significant differences emerged in the homogeneity of the distribution of cereals. Flavor attributes were ascribed to the typical characteristic of cereals and/or connected to the cooking step. The addition of the DFFF did not affect the cereal-like note. The flavor of caramel, instead, was significantly more intense in the DFFF-enriched snack bar than in the control, probably due to the thermal effect exerted by the hot glucose syrup on DFFF during the preparation of the snack bar.

Table 4. Results of the quantitative descriptive sensory analysis of the experimental snack bars. DFFF = dry-fractionated fine fraction of defatted durum wheat cake.

Sensory parameter	Type of snack bar	
	DFFF-enriched	Control
Visual		
Yellowish color	$6.1±1.2^{a}$	$4.0±0.9^{b}$
Brightness	$5.0±1.1^{b}$	$7.1±0.8^{a}$
Homogeneity of the distribution of cereals	$6.8±0.4^{a}$	$7.0±0.6^{a}$
Flavor		
Cereal-like	$6.8±0.9^{a}$	$6.8±1.3^{a}$
Toasted	$4.7±1.2^{a}$	$3.8±1.0^{a}$
Caramel	$4.1±0.7^{a}$	$2.2±1.1^{b}$
Abnormal odours	$0.2±0.4^{a}$	$0.3±0.8^{a}$
Taste		
Sweetness	$4.5±0.5^{a}$	$4.1±0.5^{a}$

Different letters indicate significant differences at $p < 0.05$.

Heat treatments of bran and similar materials are known to develop volatile compounds with sweet and caramel-like aromas (Arsa et al., 2018). Indeed, also the toasted note was perceived more intensely in the DFFF-enriched snack bar, but the difference with the control was not statistically significant. No abnormal smells were detected in either sample. The taste evaluation showed that both the snack bars had an equally perceived medium sweet taste, imputable to the glucose syrup.

Conclusions

The use of DFFF as food ingredient could favour the circularity of the durum wheat supply chain while responding to the demand for healthy and ready-to-eat food products expressed by modern consumers. The outcomes of this study demonstrated that the development of an innovative snack bar enriched of DFFF is a feasible strategy to this end.

DFFF should not be added in quantities higher than 10%, and in any case must be lower than the amount of glucose syrup, in order to ensure an optimal structure and adequate sensory acceptability of the snack bar. Nonetheless, thanks to the fortification with DFFF, the proposed snack bar showed improved nutritional characteristics, with enhanced protein content. Noteworthily, the optimized formulation can be labelled as “low fat” and “source of fiber” according to the EC Reg. 1924/06. Furthermore, being exclusively made of plant-based ingredients, without additives, the DFFF-enriched snack bar was a clean labelled food product and met the expectations of vegetarian and vegan consumers.

This study could pave the way for the formulation of other DFFF-enriched snack bars, for example by incorporating nuts and/or dehydrated fruit, to offer the consumer a wide choice of flavors and tastes while meeting the current trends in sustainable food consumption.

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AUTHOR CONTRIBUTIONS

Giacomo Squeo: Software, Formal analysis, Data curation, Writing - Original draft preparation; Vittoria Latrofa: Methodology, Investigation, Data curation, Writing - Original draft preparation; Francesca Vurro: Methodology, Investigation; Davide De Angelis: Formal analysis; Francesco Caponio: Writing - Review & Editing; Carmine Summo: Formal analysis, Writing - Review & Editing; Antonella Pasqualone: Conceptualization, Writing - Review & Editing, Supervision. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

The data used to support the findings of this study can be made available by the corresponding author upon request.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Chapter 5. Effect of durum wheat meal protein on the techno-functional, textural, volatile and sensory properties of low-moisture texturized vegetable proteins

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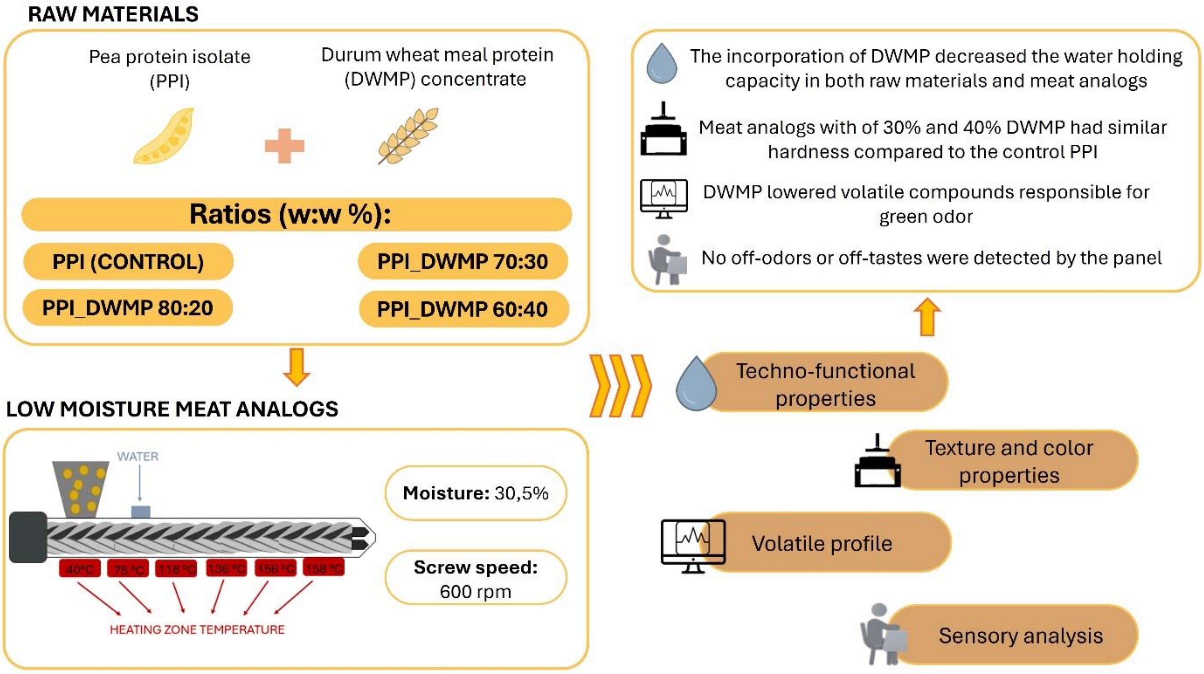
Graphical abstract



Abstract

Durum wheat meal protein (DWMP) concentrate, obtained through dry fractionation of by-products from durum wheat milling and germ oil extraction, has significant potential for valorization. Its consistent availability, large-scale production, and nutritional value make it promising for plant-based texturized vegetable proteins (TVP). In this study, low-moisture extrusion was used to study combinations of DWMP with pea protein isolate (PPI) at PPI:DWMP ratios of 80:20, 70:30, and 60:40, with 100% PPI as control. Techno-functional and sensory properties were influenced by the ratios of ingredients. DWMP addition reduced the water holding capacity in both the raw materials and TVP but increased the water solubility index. A higher DWMP ratio led to greater expansion of TVP. Samples containing 30% and 40% DWMP exhibited a hardness (3552 ± 595 g and 3144 ± 459 g, respectively) comparable to that of PPI (2959 ± 374 g), while springiness and cohesiveness were reduced. Moreover, DWMP lowered volatile compounds linked to green odors (e.g., hexanal) without introducing off-odors or off-tastes, as confirmed by sensory analysis.

These findings demonstrate that DWMP can be utilized in the formulation of TVP with satisfactory techno-functional, textural, and sensory properties, broadening the range of alternative protein sources for the food industry.

Keywords: durum wheat meal; plant-based texturized vegetable proteins; techno-functional properties; by-product valorization; low-moisture extrusion.

Introduction

Meat analogs are products designed to replicate the sensory and structural properties of animal meat. Their production involves various techniques, with extrusion cooking being one of the most widely used (De Angelis et al., 2024c). Among different extrusion methods, low-moisture extrusion is particularly suitable for producing texturized vegetable proteins (TVP) used as meat analogs (Kaleda et al., 2021; Lee et al., 2022; Van der Sman & van der Goot, 2023). During extrusion, the protein material undergoes thermal and mechanical stress caused by heating, screw rotation, and high-pressure conditions in the extruder barrel (Lee et al., 2022; Dey et al., 2021). These conditions promote protein denaturation, reorganization, and alignment, leading to the formation of a three-dimensional fibrous network that resembles muscle tissue (Kaleda et al., 2021; Lee et al., 2022).

The development of texturized vegetable proteins is driven by growing consumer demand for sustainable, ethical, and health-conscious alternatives to traditional meat consumption (De Angelis et., 2024c). In response to this demand, there is a high interest in alternative protein sources, particularly those derived from by-products, aligning with the principles of a circular economy (Zahari et al., 2023). Protein-rich ingredients derived from by-products can be used either alone or in combination with conventional raw materials. For instance, researchers have investigated the use of black gram by-products (Kamani Luithui & Meera, 2021), tomato peel (Lyu et al., 2024), rice bran (Pengjun et al., 2022; dos Reis Gasparetto Cruz et al., 2025), and hempseed pressed cake (Zahari et al., 2023) in extruded texturized vegetable proteins. Among these, Kamani Luithui & Meera (2021) demonstrated that black gram protein by-products could be used to produce TVP. The authors successfully incorporated them into plant-based patties, yielding a texture comparable to that of chicken patties. Pengjun et al. (2022) investigated the use of defatted rice bran, added in varying percentages to soy protein isolates during low-moisture extrusion, and found that it negatively affected springiness and cohesiveness, indicating a need for further optimization of textural properties. Furthermore, Reis Gasparetto Cruz et al., (2025) investigated the utilization of rice protein extracted from rice bran in the formulation of TVP, concluding that by adjusting the processing conditions it is possible to obtain suitable ingredients for development of meat alternative products. Zahari et al. (2023) extended the research on by-products by also examining the high-moisture extrusion process. Specifically, they studied the effects of extrusion parameters i.e., moisture content, screw speed, and temperature during high-moisture extrusion on hempseed press cake, producing a meat analog with uniform layers and a distinct fibrous structure (Zahari et al., 2023). Nevertheless, they recommended further research to evaluate the sensory quality of these meat analogs (Zahari et al., 2023).

Despite their potential, the use of by-products in texturized vegetable proteins remains challenging due to nutritional concerns and unfavorable sensory aspects (De Angelis et al., 2024c, Dey et al., 2021). To overcome these challenges, durum wheat meal, which is the milling by-product of durum wheat (*Triticum turgidum* L. ssp. *durum*) after germ oil extraction, has emerged as a promising alternative protein source. After dry fractionation, durum wheat meal can be transformed into a protein-rich ingredient suitable for food applications (Squeo et al., 2022; Squeo et al., 2023). A key advantage of durum wheat meal protein (DWMP) is its consistent availability throughout the year and large-scale production. Approximately 38 million tons of durum wheat grains are harvested and processed annually (Zingale et al., 2022), generating a substantial amount of nutritionally valuable by-products. Specifically, it has been reported that wheat bran represents around 20–25% of semolina production and is mostly directed to the livestock feed industry (Cuomo et al., 2024). The valorization of these by-products as secondary raw materials may provide a valuable opportunity for the food industry to diversify their products and create new formulations with commercial potential (Cuomo et al., 2024). Redesigning food systems with circular approaches is also a strategy to support the reduction of greenhouse gases emissions and land use (Simon et al., 2024). However, the processes used to stabilize by-products or extract ingredients from them significantly determine their overall environmental footprint (Thorsen et al., 2024). In this context, DWMP, which is obtained through dry fractionation, may represent a sustainable solution as dry fractionation is considered the protein extraction technology with the lowest environmental impact (Lie-Piang et al., 2023).

Pasqualone et al. (2017) reported that durum wheat by-product is rich in dietary fibers and phenolic compounds, showing high antioxidant activity. However, its sensory properties in food applications and suitability for extrusion remain largely unexplored (Squeo et al., 2024), highlighting the need for further research.

Therefore, the aim of this research was to investigate the application of DWMP in low-moisture extruded TVPs, in combination with pea protein isolate. This study aimed to evaluate the effects of varying DWMP levels on the texture, color, techno-functional properties, volatile compounds, and sensory attributes of the products. The overarching goal was to valorize DWMP as a sustainable protein source and promote the broader application in meat analog formulations.

Materials and methods

Materials

Dry-fractionated durum wheat meal protein (DWMP) concentrate was provided by Casillo Next Gen Food srl (Corato, Italy). Briefly, the wheat meal was first defatted, and then it was subjected to fine milling and air classification. DWMP contained 28.53 ± 0.02 g/100g protein, 8.53 g/100g fibers, 6.24 ± 0.01 g/100g ash, 0.81± 0.01 g/100g lipid, 55.89 ± 0.14 g/100g carbohydrates (data expressed on dry matter basis). Commercial yellow pea protein isolate (PPI) was purchased from Caremoli S.p.A (Monza, Italy) and had 82.05 ± 0.26 g/100g protein, 8.44 ± 0.32 g/100g lipid, 8.0 g/100g fibers, 1.51 ± 0.21 g/100g carbohydrates (g/100g, dry matter basis). All the nutritional compositions were determined as described in the next section, except for the data of the dietary fibers which is provided by the producer. The powder mixtures used during extrusion were 100% pea protein isolate (PPI), and pea protein isolate combined with DWMP in different ratios: 80:20, 70:30, 60:40 (PPI:DWMP w:w %).

Chemical determination of raw materials

Protein content (N × 6.25) of the raw materials was determined following the Kjeldahl method AOAC 979.09. 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 extraction solvent. Ash determination was carried out as described in the method AOAC 923.03. Carbohydrates were determined by differences from protein, lipid, fiber, and ash contents. All the determinations were carried out in triplicate.

Low-moisture extrusion cooking

The extrusion cooking was performed with a co-rotating intermeshing twin-screw extruder KETSE 20/40 (Brabender GmbH, Duisburg, Germany). The processing conditions and the recipes used in the experiments and the proportions of PPI:DWMP were chosen according to the extrudability of the materials in preliminary trials and to our previous experience with similar raw materials (Kaleda et al., 2021). Specifically, a minimum inclusion level of 20% was chosen, as lower quantities were not considered valuable for the intended purpose of by-product valorization. Higher levels were subsequently tested to assess their impact on product quality. However, formulations containing DWMP above 40% were unsuitable for extrusion under the selected conditions, resulting in poor textural outcomes and processing difficulties. The temperature profile was set to 40/75/118/136/156/158 °C, the screw speed to 600 rpm. Water was added with a calibrated peristaltic pump. The diameter of the round die at the barrel exit was 1.6 mm. The extrudates were collected after the stabilization of the process, when the temperature, die pressure, and screw torque recorded every second by the extruder were constant. The samples exited the extruder as continuous strands, and they were manually broken into pieces of 4-5 cm. Then, they were dried in the oven for 50 min at 70 °C until water activity reached a value below 0.6 to prevent microbial growth. Finally, the samples were packed into zip-lock plastic bags and stored at room temperature.

Extrudates had a moisture content of 2.65 ± 0.11 g/100g after drying. The mass flow rate was calculated by collecting the extrudate for two minutes and then recording the mass of water, the mass of dried extrudate, the moisture content of the raw materials, and the moisture content of the extrudate after drying. The specific mechanical energy (SME) was calculated as reported in Kaleda et al. (2021).

Specific volume, techno-functional and textural properties of extrudates

Specific volume was performed as described in De Angelis et al. (2020) following the rapeseed displacement AACC method 10-05.01. The specific volume (mL/g of product) corresponded to the volume of the rapeseed in excess on the weight of the sample. Measurements of water holding capacity (WHC), water solubility index (WSI), and oil holding capacity (OHC) were conducted on the raw ingredients and extrudates, as described by Kaleda et al. (2021). Specifically, the WHC and the WSI were performed on the whole extrudates. For the determination of OHC and the other analyses requiring milled extrudate, the extrudate was milled with a coffee mill, then sieved with a 0.6 mm sieve. WHC was performed by suspending 1 g of whole TVP in 10 mL of distilled water at 60 °C and stirred for 60 minutes at room temperature. OHC was performed by suspending 1 g of the milled sample in 10 mL of rapeseed oil and stirred for 30 min. Then the sample was centrifuged at 3000 × *g* for 15 min at 25 °C, oil/water supernatant was decanted, and the remaining pellet weighed. WHC/OHC was expressed as the weight of water/oil in grams held by 1 g of dry sample. The water supernatant was collected and lyophilized to calculate WSI, expressed as the percentage of dry solids in the supernatant to the initial TVP weight.

Texture profile analysis (TPA) was performed on the extrudates by using a TA.XTplusC Texture Analyzer (Stable Micro-Systems, Godalming, UK) equipped with a 75 mm compression plate. Before the analysis, the extrudate samples were rehydrated in tap water at 60 °C for 60 minutes and then blotted dry. A total of 1.6 g of extruded pieces was compressed twice using a 50 kg load cell, a test speed of 1 mm/s test, until 70% compression, and a time of 2 s between the two compressions. Hardness (g), resilience (%), cohesion, springiness (%), and chewiness were calculated with the texture analyzer software.

The instrumental color of the powder ingredients and milled extrudates was measured using an NS810 Spectrophotometer (3nh Shenzhen Threenh Technology Co., Ltd., Shenzhen, China) set to illuminant D65 and 2° angle. For the analysis, the extrudates were milled using a coffee mill and sieved through a 0.9 mm sieve. The color properties were determined according to the CIE (International Commission on Illumination) LAB color space, evaluating lightness (L*, from black to white), redness (a*, from green to red), and yellowness (b*, from blue to yellow). Total color difference (ΔE) was calculated as (De Angelis et al., 2023b):

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

The following scale was considered: ΔE = 0–1, observer does not notice the difference; 1–2, only experienced observer can notice the difference; 2–3.5, unexperienced observer also notices the difference; 3.5–5, clear difference in color is noticed; > 5, observer notices two different colors (Mokrzycki & Tatol, 2011).

Protein digestibility

In vitro protein digestibility (IVPD) of the raw ingredients and milled extrudates was determined following the method described in Espinosa-Ramírez et al. (2018) with some modifications. Briefly, 1 mL of protein dispersion containing 6.25 mg of protein was heated 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 with a trypsin activity of 13,766 BAEE (Nα-Benzoyl-L-arginine ethyl ester) units/mg protein was prepared using trypsin (Sigma-Aldrich-T8003) at a concentration of 1.6 mg/mL. 0.1 mL of the enzyme solution was added to the protein suspension in the water bath, and the pH drop was recorded after 10 minutes. The IVPD percentage was calculated using the equation $y = 210.464 - 18.1\,x$, where x is the pH value after 10 minutes (Espinosa-Ramírez et al., 2018).

Volatile profile

Volatile compounds of raw materials and of dry extrudates were extracted using solid phase microextraction (SPME). A total of 0.3 g of sample and 0.7 mL of distilled water was measured into a 10 mL headspace vial (glass vials 10 mL screw neck, Shimadzu) by using an equilibration temperature of 40 °C and inlet temperature of 250 °C. A SPME fiber (30/50µm DVB/Car/PDMS Stableflex, 1 cm long) was utilized to absorb volatile compounds from the headspace (HS) over a period of 40 minutes. Subsequently, the absorbed volatile compounds were desorbed into a GC injection port over 5 minutes.
The analysis was performed using a Shimadzu 2030 gas chromatography system coupled with an 8050NX Triple Quadrupole mass spectrometer (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 utilized with helium as the carrier gas, flowing at a linear velocity of 35 cm/s. The oven temperature was programmed to increase from 40 °C at a rate of 5 °C/min to 180 °C followed by temperature ramp of 25 °C/min until reaching 280 °C, with an additional hold time of 3 minutes, resulting in a total run time of 36.6 minutes. Mass spectra were generated at an ionization energy of 70 eV with a mass-to-charge ratio scan range of 35 to 350. Compound identification was confirmed by comparing the experimental retention indices with the ones in the NIST17 and FFNSC libraries. A semi-quantitative method was applied, using 4-methyl-2-pentanol as an internal standard to quantify the identified volatile compounds in terms of IS ppb-equivalents.

Sensory analysis

Descriptive sensory analysis was conducted in AS TFTAK laboratories (Tallinn, Estonia) by an internal trained sensory panel, providing repeatability and reliability of sensory tests (Djekic et al., 2021; Sipos et al., 2021). All assessors were recruited from AS TFTAK (Tallinn, Estonia) in accordance with ISO 8586:2012 standard, following the prescribed procedures for the training and monitoring of expert sensory assessors. Ten trained assessors (average age 33 ± 9 years) with prior general training and additional experience in evaluating plant-based extrudates were selected for the study. Prior to the assessment sessions, an additional training session was conducted to allow the assessors to familiarize themselves with these samples and to revise the evaluation methods, including the use of the scale.

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. The research followed the protocols designed to protect the rights, privacy, and welfare of participants. Written consent was obtained from all panelists, and participation was voluntary, allowing panelists to withdraw at any time. The selected panelists were informed in advance of the purpose of the study and its procedures. All participants were in good health and had no known allergies to the components. Participants were assured of the confidentiality of their data.

Table 1. Sensory descriptors and their definition, based on the TFTAK's in-house developed protocol.

Attribute	Definition	Technique
Odor		
Overall intensity	Perceived odor intensity of the sample	Sniffing
Raw material	Associations with grains and legumes (e.g., wheat, pea)	Sniffing
Roasty	Perceived roasty nuances (e.g., burnt, cooked, toasty)	Sniffing
Off-odor intensity	Non-characteristic odors (i.e., chemical, rancid, metallic, earthy)	Sniffing
Taste		
Overall intensity	Perceived taste intensity of the sample	Tasting
Raw material	Associations with grains and legumes (e.g., wheat, pea)	Tasting
Umami	Perceived intensity of umami taste in the sample (i.e., meaty, brothy)	Tasting
Bitter	Perceived bitterness similar to caffeine and quinine	Tasting
Astringency	Perceived astringency which causes a dry, sharp, mouth puckering feeling	Tasting
Off-taste intensity	Non-characteristic taste (i.e., chemical, rancid, metallic, earthy)	Tasting
Texture		
Fibrousness	Refers to long particles oriented in the same direction	By hands
Springiness	Mechanical textural attribute relating to the rapidity of recovery from a deforming force and degree to which a deformed material returns to its undeformed condition after the deforming force is removed	Mouthfeel
Cohesiveness	Characterizes the strength of the internal bond i.e., the amount of sample that holds together when chewed	Mouthfeel
Adhesiveness	Mechanical textural surface attribute relating to the force required to remove material that adheres to the mouth	Mouthfeel
Moistness	The perceived amount of water in the sample released during chewing	Mouthfeel
Granularity	Granularity is the extent to which the sample contains or induces granular mouthfeel	Mouthfeel

Assessments were held individually in isolated testing booths in a dedicated sensory room (in accordance with ISO 8589:2007). Sensory analysis was conducted in two replicate sessions, with each sample presented once per session (totaling four samples per session). A 2-hour break was provided between sessions to mitigate sensory fatigue. Dry extrudate samples were rehydrated in tap water at 60 °C for 60 minutes and then blotted with paper towels to remove the excess water. 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 (MacFie et al.,1989). Five grams of the rehydrated samples were served to assessors at room temperature (21 °C) in clear 40 mL plastic cups and presented in sequentially monadic order.
The sensory analysis was conducted according to an internal sensory protocol, evaluating odor, taste, and texture. All texture attributes and their definitions, reported in Table 1, were based on TFTAK's in-house developed protocol. Quantitative descriptive analysis used a 10-point structured scale, where 0—"none", 1—"very weak", 5—"moderate", and 9—"very strong". To clean the palate between the samples, the assessors were served spring water and unflavored crackers on the side at the time of assessment. Data collection was conducted using RedJade sensory software version 6.1 (RedJade Sensory Solutions LLC, Martinez CA, USA).

Statistical analysis

All the data are expressed as a mean and standard deviation. The means of the samples were processed with one-way analysis of variance ANOVA followed by Tukey's HSD (Honestly Significant Differences) test for multiple comparisons at a significance level α = 0.05 by using Minitab 19 Statistical Software (Minitab Inc., State College, PA, USA), except for volatile profile data which was processed using pairwise t-tests by using the same statistic software. WHC, WSI, OHC, specific volume, color, amino acids, volatile profile, and IVPD were measured in triplicate, TPA in eight replicates, and the sensory analysis was done in two replicate sessions. The figures were made using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA).

Results and discussion

Extruder responses during TVP production

The recorded parameters during low-moisture extrusion are presented in Table 2, whereas Figure 1 shows the twisted elongated appearance of the produced TVPs.

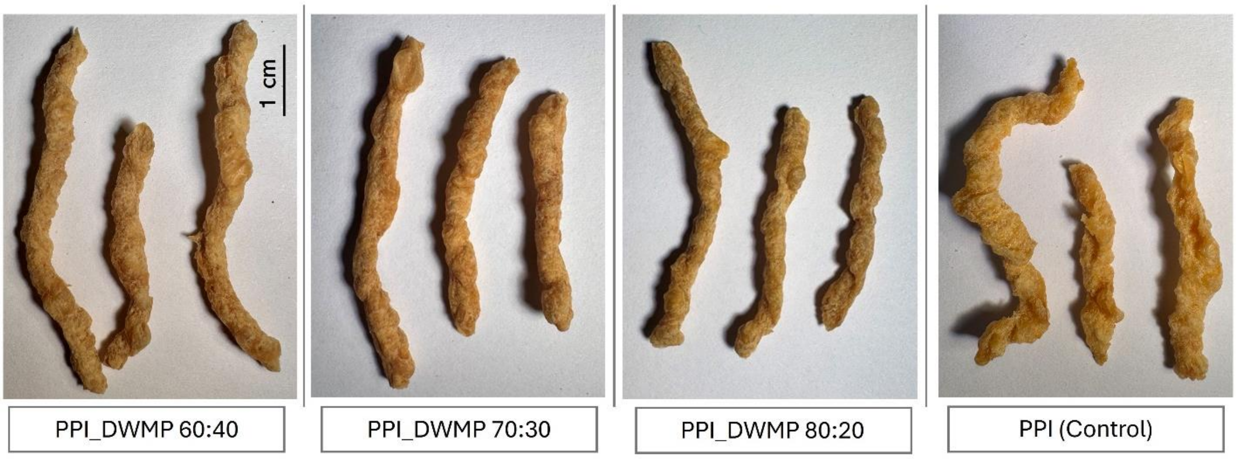


Figure 1. Visual appearance of texturized vegetable protein (low-moisture meat analogs) produced from pea protein isolate (PPI) and durum wheat meal protein (DWMP) blends.

Extrusion responses can be generally linked to the physicochemical reactions of the macromolecules that undergo at specific extrusion conditions, and these reactions are responsible for the structure formation (De Angelis et al., 2023a; Opaluwa et al. 2025). The TVPs containing DWMP showed some differences between the extrudates. As the DWMP content increased, the powder flow rate decreased due to changes in the powder density and flow behavior in the volumetric feeder. The die pressure and motor load also lowered. Indeed, when the die pressure is high, the screw torque could also increase to push through the melt in the extruder (Zheng et al., 2024). While die pressure is dependent on the flow rate (Opaluwa er al., 2025), it can also be correlated to the melt viscosity (De Angelis et al., 2023a; Opaluwa et al. 2025), which depends on other parameters including moisture content, ingredient water absorption properties, temperature, and screw speed. Product temperature decreased slightly while the SME showed the opposite behavior. Nevertheless, the differences in extrusion conditions were relatively small and the samples were broadly comparable.

Table 2. Extrusion parameters of the TVPs produced.

	PPI	PPI_DWMP 80:20	PPI_DWMP 70:30	PPI_DWMP 60:40
Total feed rate (kg/h)	5.11	4.83	4.61	4.45
Dry matter feed rate (kg/h)	3.43	3.17	2.97	2.82
Water feed rate (kg/h)	1.68	1.66	1.64	1.63
Total moisture content of the melt in the barrel (%)	32.80	34.50	35.60	36.60
Die pressure (bar)	15.70 ± 0.41a	13.64 ± 0.45b	12.72 ± 0.37c	11.54 ± 0.44d
Motor load (%)	18.38 ± 1.92ab	18.77 ± 1.91a	18.24 ± 2.03b	17.98 ± 1.83b
Product temperature (°C)	150.85 ± 0.14d	151.85 ± 0.05a	151.79 ± 0.03b	151.59 ± 0.03c
SME (Wh/kg)	133	147	145	146

PPI—Pea Protein Isolate; DWMP—Durum Wheat Meal protein; SME—Specific mechanical energy. Data are expressed as a mean ± SD (n = 241). Different letters in the same row indicate significant differences according to Tukey's HSD test ($p \leq 0.05$).

Techno-functional properties and protein digestibility of the raw materials and extruded TVPs

Techno-functional properties of food ingredients embrace all characteristics, excluding their nutritional value, that influence their behavior during food processing and preparation, thereby determining their technological role in the final product (Mshayisa et al., 2022; De Angelis et al., 2024b).

The techno-functional properties of raw materials and extrudates are reported in Table 3. In the raw materials the substitution of PPI with DWMP resulted in a gradual decrease in WHC and an increase in WSI. The OHC remained unaffected, and the values were consistent with previous studies on plant protein ingredients (Silventoinen et al., 2021; Jakobson et al., 2023; De Angelis et al., 2024b). WHC and OHC indicate the ability of an ingredient to bind water or oil per gram, whereas WSI describes powder solubility at room temperature and native pH (De Angelis et al., 2024a; Jakobson et al., 2023). Considering the raw material composition, it can be assumed that the mechanism of water retention was mainly attributed to proteins (Schlangen et al., 2022), as increasing the DWMP ratio led to a decrease in protein content and, consequently, a reduction in WHC. Similar findings were previously reported by Schlangen et al. (2022). At the same time, the increase in WSI may be attributed to carbohydrates present in DWMP, which are more soluble (De Angelis et al., 2024a).

Similar trends in WHC, WSI, and OHC were observed in both raw materials and TVPs, suggesting that analyzing the properties of raw materials prior to extrusion could provide insights into the characteristics of the final product. WHC and WSI were analyzed in whole extrudates to investigate the physical entrapment of water and to simulate their application in food formulations after rehydration (Lee et al., 2022). Previous studies suggest that WHC may play a crucial role in the sensory attributes of a TVP, influencing juiciness, tenderness, texture, and mouthfeel (Lee et al., 2022; Brishti et al., 2021). However, sensory analysis found no significant differences in moistness, indicating that the WHC reduction was minor and not perceptible to the expert panel. This implies that WHC is primarily important from a technological perspective, enabling the understanding of water absorption behavior in texturized proteins during meat alternative product preparation. Similar values of WHC were previously observed in TVP produced from rice protein (Gao et al., 2022). After extrusion, WSI decreased possibly due to protein denaturation and aggregation, which can also reduce the solubility of carbohydrates entrapped in the matrix structure (Kaleda et al., 2021). Indeed, Gao et al. (2022) evaluated the secondary structure of low-moisture extrudates finding that that the extrusion process induced changes in chemical bonds and forces between the molecules, which modify the protein structure. OHC increased by approximately 20% in the TVPs compared to the raw materials. However, no significant differences were found among the extrudates. This increase was likely due to protein denaturation during the extrusion process, which exposes hydrophobic groups, allowing greater oil binding (Kaleda et al., 2021; Zhang et al., 2023). The absence of significant differences among the TVPs suggests that OHC was mainly influenced by physical oil entrapment within the porous microstructure (Kaleda et al., 2020), as the analysis was performed on milled extrudates. Similar OHC values have been reported by Kamani et al. (2021) and Gao et al. (2022) in TVPs produced from black gram by-product and rice protein, respectively. Protein digestibility is generally associated with amino acid availability and the sequence of amino acids within the polypeptide chain (Espinosa-Ramírez et al., 2018), and it varies depending on the raw material composition, genetic and environmental factors, and the presence of anti-nutritional compounds. Typically, protein digestibility ranges from 70% to 80% (Espinosa-Ramírez et al., 2018), when measured by the same method of analysis as used in this study. The protein digestibility of the raw materials before extrusion showed no significant differences with the addition of DWMP to PPI (Table 3). IVPD was also evaluated in the extrudates to assess the impact of the extrusion process on protein digestibility (Table 3).

After extrusion, IVPD significantly increased in all samples by an average of 14%, consistent with values reported for legume extrudates by other authors (Palanisamy et al., 2019). The increase in IVPD can be attributed to the high temperature and mechanical action of the extruder screws, which induce structural changes in proteins, such as unfolding, that expose cleavage sites to enzymes, thereby improving digestibility (Beck et al., 2017). Indeed Wang et al. (2020) reported that the extrusion process changes the protein conformation from α-helix to β-sheet leading to an improved protein digestibility. Furthermore, the enhanced IVPD may be linked to the inactivation of anti-nutritional factors during extrusion (i.e., trypsin and chymotrypsin inhibitors) (Beck et al., 2017; Rathod & Annapure, 2016).

Table 3. Techno-functional properties and protein digestibility of the raw materials and TVPs expressed on a dry weight basis.

	Raw materials				TVPs			
Parameter	PPI	PPI_DWMP 80:20	PPI_DWMP 70:30	PPI_DWMP 60:40	PPI	PPI_DWMP 80:20	PPI_DWMP 70:30	PPI_DWMP 60:40
WHC (g water/g material)	3.01 ± 0.13a	2.55 ± 0.09b, *	2.39 ± 0.09b, *	2.11 ± 0.00c, *	2.82 ± 0.18a	2.14 ± 0.13b, *	1.80 ± 0.02c, *	1.75 ± 0.04c, *
WSI (%)	10.78 ± 0.48c, *	14.63 ± 0.56b, *	15.69 ± 0.71ab, *	16.35 ± 0.28a, *	9.32 ± 0.45c, *	10.53 ± 0.44b, *	11.72 ± 0.07a, *	12.34 ± 0.52a, *
OHC (g oil/g material)	1.03 ± 0.04a, *	0.95 ± 0.07a, *	0.97 ± 0.03a, *	0.99 ± 0.03a, *	1.23 ± 0.04a, *	1.27 ± 0.01a, *	1.21 ± 0.03a, *	1.20 ± 0.03a, *
IVDP (%)	75.08 ± 0.48a, *	72.48 ± 1.06a, *	73.99 ± 1.63a, *	72.48 ± 0.93a, *	85.94 ± 0.48a, *	84.97 ± 0.86ab, *	85.82 ± 0.28a, *	83.82 ± 0.28b, *

PPI—Pea Protein Isolate; DWMP—Durum Wheat Meal protein; WHC—Water Holding Capacity; WSI—Water Solubility Index; OHC—Oil Holding Capacity; IVPD—In Vitro Protein Digestibility. Data are expressed as a mean ± SD (n = 3). Different letters in the same row indicate significant differences according to Tukey's HSD test ($p \leq 0.05$) within the same category, either raw materials or TVPs; * indicates significant differences between the TVP and the corresponding raw material.

The PPI_DWMP 60:40 extrudate was the only sample with a slightly significantly lower IVPD than the control PPI extrudate. This may be due to the presence of carbohydrates and dietary fibers from DWMP, which can reduce protein digestibility by inhibiting enzymatic hydrolysis (Liu et al., 2023a). In fact, based on the data of the proximate composition, PPI_DWMP 80:20, PPI_DWMP 70:30, and PPI_DWMP 60:40 had 12.39, 17.82, and 23.26 g/100g of carbohydrates, respectively, compared to the control PPI which had only 1.51 g/100g of carbohydrates. Carbohydrates and fibers can interact with proteins through hydrogen bonding and van der Waals forces, altering or inhibiting enzyme activity and thereby reducing protein digestion efficiency (Liu et al., 2023a).

Specific volume, texture and color properties

The substitution of DWMP for PPI significantly increased the specific volume of the extrudates (Table 4) when DWMP content was at least 30%. This effect could be attributed to the higher carbohydrate content in DWMP, which enhances expansion. It has been reported (Zhang et al., 2019) that during the texturization of low-moisture extrudates, carbohydrates and starch granules tend to expand at the die exit due to the conversion of water into vapor. Conversely, the higher density observed in PPI and PPI_DWMP 80:20 extrudates may be attributed to their higher protein content, which promotes cross-linking between proteins, restricting expansion (Brishti et al., 2021).

The texture profile analysis of TVPs is reported in Table 4. Hardness significantly increased only in the sample PPI_DWMP 80:20, whereas in the other formulations it remained similar to the control PPI. The results may be explained by the interaction of two different factors: the water absorption and the expansion of the extrudates. The analysis was conducted on rehydrated products and the amount of absorbed water influences hardness (Lee et al. 2022). Furthermore, previous studies (Lee et al., 2022; Kaleda et al., 2021; do Carmo et al., 2023) also have reported that samples with higher porosity, which typically indicates higher expansion, also tend to exhibit lower hardness values. Consequently, although DWMP decreased water absorption, the higher expansion observed in samples containing 30% and 40% DWMP counteracted this effect, leading to a lower hardness in those extrudates. This outcome is consistent with the Texture Profile Analysis (TPA), a compression-based test, in which increased expansion and the presence of air bubbles within the extrudates may have contributed to reduced resistance during compression.

Adhesiveness increased with higher DWMP content, while resilience, cohesion, and springiness decreased. Chewiness decreased only in the PPI_DWMP 60:40 sample. Based on the composition of the raw materials, the increased adhesiveness at higher DWMP levels may be linked to the presence of carbohydrates, including sugars and starch, which is commonly used as a binding agent in meat alternatives (Kyriakopoulou et al., 2021). The improved adhesiveness of DWMP extrudates could be beneficial, as it may reduce the need for additional binder ingredients, such as methylcellulose (Kyriakopoulou et al., 2021). Similar cohesion values were observed in TVP produced from rice by Lee et al. (2022).

Springiness represents the extent to which a sample recovers its shape after deformation, whereas resilience measures the speed of this recovery (Kaleda et al., 2021). As DWMP content increased, springiness decreased, likely due to lower protein content and subsequent reduced bond formation during texturization (Kaleda et al., 2021; Brishti et al., 2021). Furthermore, springier foods require more effort to chew and break down in the mouth (Kaleda et al., 2021), making the reduction in springiness in samples with 30% and 40% DWMP a potentially positive result. Springiness influences cohesion, which reflects the internal structural strength of a material (Lee et al., 2022). Cohesion depends on protein content and cross-linking between proteins, typically higher at higher protein levels (do Carmo et al., 2023). Similarly, chewiness decreased in extrudates with higher DWMP levels. This reduction in chewiness may be due to the higher carbohydrate content in DWMP, leading to a more porous and less chewy structure (do Carmo et al., 2023). The findings on specific volume further support this result. Since chewiness is a complex parameter related to both hardness and moistness (Kaleda et al., 2021) and is critical for the acceptability of TVPs, it can be hypothesized that DWMP-based TVPs may be easier to eat than the control PPI formulation.

Table 4. The specific volume, textural and color properties of TVPs produced with PPI and DWMP at different ratios.

Parameter	PPI	PPI_DWMP 80:20	PPI_DWMP 70:30	PPI_DWMP 60:40
Specific volume (mL/g)	2.23 ± 0.07b	2.40 ± 0.04b	2.83 ± 0.09a	2.71 ± 0.08a
Hardness (g)	2959 ± 374b	4029 ± 492a	3552 ± 595ab	3144 ± 459b
Adhesiveness (g*sec)	-0.06 ± 0.24a	-1.21 ± 1.36ab	-2.5 ± 0.44b	-8.45 ± 2.35c
Resilience (%)	23.62 ± 2.78a	22.57 ± 2.33a	19.14 ± 1.65b	13.37 ± 1.49c
Cohesion (-)	0.57 ± 0.05a	0.56 ± 0.05a	0.5 ± 0.04b	0.40 ± 0.05c
Springiness (%)	57.26 ± 7.96a	53.35 ± 7.64ab	45.02 ± 3.38bc	41.67 ± 8.16c
Chewiness (-)	951 ± 164b	1201 ± 233a	763 ± 106bc	527 ± 133c
*L**	77.95 ± 1.15a	74.82 ± 0.44b	75.67 ± 0.30b	75.08 ± 0.31b
*a**	2.19 ± 0.03b	3.21 ± 0.16a	3.24 ± 0.06a	3.36 ± 0.12a
*b**	23.26 ± 0.42a	22.73 ± 0.12ab	22.16 ± 0.39b	22.09 ± 0.05b
ΔE	-	2.06 ± 0.07	1.40 ± 0.44	1.65 ± 0.38

PPI—Pea Protein Isolate; DWMP—Durum Wheat Meal protein; L*—lightness; a*—redness; b*—yellowness. Data are expressed as a mean ± SD (*n* = 8 for TPA and *n* = 3 for color). Different letters in the same row indicate significant differences according to Tukey's HSD test ($p \leq 0.05$).

Color properties of extrudates are presented in Table 4. Lightness (*L**) did not vary between the DWMP-containing extrudates but was lower compared to the control PPI. Conversely, the samples with DWMP exhibited higher redness (*a**) and lower yellowness (*b**) compared to the control PPI, except for PPI_DWMP 80:20, which had a *b** value similar to the control PPI. Comparable results were found by other authors (Lee et al., 2022; Kaleda et al., 2020). Non-enzymatic reactions during extrusion, i.e., Maillard reaction and caramelization, modify the color of the raw material (Lee et al., 2022). Due to the high temperature, moisture, friction, and pressure, proteins are converted into amino acids and starch is reduced to sugars (Lee et al., 2022). The Maillard reaction involves chemical reactions between free amino acids and reducing sugars, producing melanoidins, whereas the caramelization reaction requires only sugars (Lee et al., 2022; Wang et al., 2022). Melanoidins are responsible for pigment formation, conferring an intense color to the product, while browning compounds are formed during the caramelization reaction (Schutte et al., 2024). Thus, it is reasonable to assume that the higher carbohydrate content in DWMP-containing formulations promoted pigment formation, leading to decreased lightness and increased redness. ΔE values were calculated to determine whether these differences were perceptible in the samples. Because ΔE ranged from 1.4 to 2, this indicates that only a trained observer could detect the color variation. This is relevant, as color is a key factor influencing consumer acceptance. Consequently, the impact of DWMP on color can be considered negligible.

Volatile profile of raw materials and TVPs

The volatile profiles of the initial raw materials and the extrudates showed many differences in the concentrations of odor-active compounds. Volatile compounds were evaluated both in the raw material and in the TVP to assess the impact of the extrusion process. A total of 82 volatile compounds from 11 classes were detected. Of these, 35 compounds with the highest concentrations and relevance for meat alternatives according to literature (Wang et al., 2022; Deavaere et al., 2022; Yuan et al., 2023) are presented in Table 5.

The enzymatic oxidation of free fatty acids and degradation of amino acids are key pathways in the production of volatile compounds in plant-based ingredients, such as pulses and cereals. These reactions may occur during both protein extraction and storage (Zhogoleva et al., 2023; Heiniö et al., 2016). In the raw materials, lipoxygenase catalyzes the degradation of linolenic and linoleic acids, with consequent formation of several odor-active compounds (Zhogoleva et al., 2023; Heiniö et al., 2016). Other fatty acids that contribute to the formation of aroma compounds are oleic acid and arachidonic acid (Zhogoleva et al., 2023). The total content of alcohols was statistically similar across all raw materials, while ketones decreased with the addition of DWMP. Aldehydes, alcohols, and ketones are major volatile compounds in pea protein, typically formed through lipid oxidation and enzymatic reactions and are associated with off-flavors such as green and vegetable odors (Zhogoleva et al., 2023; Wang et al., 2022). Among alcohols, 1-octen-3-ol is particularly important, as it contributes to beany flavors in meat alternatives and is characteristic of pea protein, resulting from the autooxidation of linolenic and linoleic acids (Jiang et al., 2024; Zhogoleva et al., 2023). In the TVPs, both alcohols and ketones decreased compared to the raw material, and increasing DWMP content led to a progressive reduction of these compounds. The high temperatures used during extrusion promote fatty acid oxidation, accelerating their conversion into other volatiles, thus decreasing the alcohol and ketone content (Yuan et al., 2023). Given the low lipid content of DWMP, its addition may have further limited their formation.

Aldehydes, mainly derived from oxidation processes (Yuan et al., 2023), were the most abundant compound class, consisting of 10 different volatiles, with hexanal, pentanal, and benzaldehyde being the most prevalent. In raw materials, these three aldehydes were the main detected aldehydes, and their concentrations were significantly higher in PPI than in all formulations containing DWMP, consistent with reports on key aroma compounds in pea proteins (Liu et al., 2023b; Zhogoleva et al., 2023). Hexanal is one of the most significant contributors to the undesirable green and beany odor, having one of the lowest detection thresholds in water (0.0045 ppb) (Yuan et al., 2023), particularly in foods containing pea or other legume proteins (Liu et al., 2023b). After extrusion, aldehyde concentrations increased in all DWMP-containing samples compared to the raw materials but remained lower than in the extruded control PPI. This increase was expected, as aldehydes can form through fatty acid degradation, the Maillard reaction, or Strecker degradation during thermo-mechanical processing like extrusion-cooking (Feng et al., 2024). The higher carbohydrate content in DWMP likely contributed to aldehyde formation via the Maillard reaction. Notably, the lower aldehyde concentration in DWMP-containing samples may be beneficial for meat alternative formulations, as reducing beany and green flavors can enhance consumer acceptance (Wang et al., 2022; Vurro et al., 2024). However, the sensory analysis found no significant differences in odor perception, indicating that these variations were not detectable by the panelists.

The addition of DWMP resulted in higher concentration of acetic acid and methyl ester, especially before extrusion. After extrusion, the control PPI sample exhibited an increase in this ester, whereas the sample containing DWMP showed a decrease, albeit with significantly higher levels than the PPI extrudate. Ester formation in grains occurs through non-enzymatic esterification reactions between alcohols and organic acids (Yuan et al., 2023). While esters contribute to fruity flavors, their limited concentration suggests a minor impact on overall aroma (Qiang et al., 2025).

Toasty, caramelized, and roasted aromas, commonly found in heat-treated foods, arise from furans and pyrazines (Yuan et al., 2023). These compounds are generated during the manufacturing of plant-based ingredients (e.g., during drying) and are increased during extrusion, due to the intense thermomechanical treatment (Wang et al., 2022; Zhogoleva et al., 2023). Among pyrazines, only 2,5-dimethylpyrazine was detected, with higher levels in DWMP-containing raw materials. Pyrazines are mainly formed through Strecker degradation in the Maillard reaction (Yuan et al., 2023). Due to their very low sensory perception thresholds, pyrazines are highly detectable (Liu et al., 2023b). Both DWMP and PPI underwent heat treatments during raw material processing but considering that durum wheat meal was defatted and dried before dry fractionation, this process may have led to greater pyrazine development compared to PPI. Nevertheless, after extrusion, pyrazine levels in DWMP-containing samples became comparable to those in the control PPI.

Among furans, 2-pentylfuran was the most prevalent in raw materials, with its concentration decreasing as the content of DWMP increased. This compound is known to form through oxidation and photooxidation of lipids due to storage conditions, heat, light exposure, and drying (Yang et al., 2023), particularly in the production of legume proteins (Zhogoleva et al., 2023). As expected, extrusion led to a significant increase in furans across all formulations compared to the raw material. Furans are generated through various pathways, including the Maillard reaction, thermal degradation of carbohydrates, and oxidation of fatty acids and carotenoids (Yuan et al., 2023; Zhogoleva et al., 2023). Given the complexity of the reaction pathways involved, it is difficult to determine which mechanisms were most influential. Additional sensory insights related to roastiness are discussed in the next section.

Total sulfur compound levels were lower in DWMP-containing samples than in the control PPI, both in raw materials and extrudates. Among sulfur compounds, methanethiol was the most abundant and its concentration decreased with DWMP addition. By contrast, thiophene and thiophene, 2-hexyl-, which were not found in the raw materials, appeared in the TVPs. In fact, thiophene can be generated during extrusion from cysteine (Jiang et al., 2024). Zhogoleva et al. (2023) identified methanethiol as a potent odor-active compound in pea protein isolates, significantly impacting flavor. Methanethiol, along with hexanal and 1-octen-3-ol, is one of the most impactful contributors to vegetable-like odor (Rashwan et al., 2023; Jiang et al., 2024; Zhogoleva et al., 2023).

Overall, while DWMP had a positive impact on the volatile profile of TVPs by reducing compounds associated with undesirable flavors, such as aldehydes, alcohols, and methanethiol, it is essential to acknowledge that volatile compounds have varying odor perception thresholds (Wang et al., 2022). Consequently, these differences may be indistinguishable to consumers, necessitating sensory evaluation.

Table 5. Semi-quantitative concentrations of 37 selected volatile compounds. Concentrations are given in µg/kg, all the odor descriptions are taken from The Good Scents Company Information System (Luebke, 1980).

			Raw materials				TVPs			
Compound	**Odor description**	**RI exp.**	PPI	PPI_DWMP 80:20	PPI_DWMP 70:30	PPI_DWMP 60:40	PPI	PPI_DWMP 80:20	PPI_DWMP 70:30	PPI_DWMP 60:40
Alcohols										
1-Hexanol, 2-ethyl-	Citrus, fresh, floral, oily	1001	5.4 ± 0.3b,*	5.7 ± 0.3b	7.2 ± 1.0a,*	5.6 ± 0.7b,*	9.7 ± 0.1a,*	9.7 ± 1.9a	11.2 ± 1.0a,*	9.5 ± 0.4a,*
1-Penten-3-ol	Ethereal, horseradish, green, radish	653	86.7 ± 9.6a,*	77.3 ± 2.0ab,*	68.1 ± 4.5b,*	67.6 ± 5.0b,*	52.2 ± 6.3a,*	33.5 ± 2.5b,*	19.0 ± 1.4c,*	11.8 ± 1.2d,*
1-Pentanol	Fusel, oily, sweet, balsamic	739	179.3 ± 17.6a,*	129.0 ± 15.0b,*	120.4 ± 21.0b,*	113.2 ± 18.2b,*	113.9 ± 8.1a,*	69.8 ± 5.4b,*	59.6 ± 1.2b,*	38.3 ± 3.5c,*
1-Hexanol	Ethereal, fusel, oily, fruity	844	82.1 ± 4.8b,*	135.2 ± 0.0a,*	176.1 ± 24.9a,*	161.3 ± 31.3a,*	43.6 ± 1.1ab,*	45.4 ± 7.9ab,*	51.2 ± 1.3a,*	39.2 ± 3.6b,*
1-Octen-3-ol	Mushroom, earthy, green, beany, oily	953	138.5 ± 19.1a	105.9 ± 6.2b	127.1 ± 7.5ab,*	125.6 ± 14.9ab,*	115.6 ± 9.0a	83.8 ± 14.5bc	94.4 ± 5.1b,*	76.8 ± 3.0c,*
Total			492.0 ± 28.1a,*	453.3 ± 16.3a,*	498.9 ± 33.7a,*	473.4 ± 39.5a,*	335.0 ± 13.6a,*	242.1 ± 17.6b,*	235.4 ± 5.7b,*	175.6 ± 6.0c,*
Aldehydes										
Butanal, 3-methyl-	Ethereal, aldehydic, chocolate, peach	629	4.9 ± 0.5c	5.0 ± 0.3c,*	6.8 ± 0.8b,*	9.0 ± 0.9a,*	5.2 ± 0.8c	14.7 ± 1.2b,*	20.4 ± 1.9a,*	21.4 ± 0.6a,*
Butanal, 2-methyl-	Musty, cocoa, phenolic, coffee	637	3.4 ± 0.6a	2.1 ± 0.0b,*	2.8 ± 0.3ab,*	3.0 ± 0.4a,*	3.9 ± 0.2c	8.4 ± 1.3b,*	10.2 ± 0.4a,*	11.3 ± 0.9a,*
Pentanal	Fermented, bready, fruity, nutty	667	454.4 ± 19.1a,*	198.3 ± 10.3b,*	146.2 ± 10.7c,*	120.7 ± 11.9c,*	407.9 ± 18.1a,*	319.4 ± 19.0b,*	260.9 ± 23.4c,*	215.6 ± 15.1d,*
Hexanal	Grass, green, beany	775	1518.1 ± 79.8a,	611.1 ± 36.7b,*	353.4 ± 54.0c,*	287.0 ± 48.8c,*	1441.0 ± 42.4a	1337.2 ± 66.4b,*	1160.1 ± 22.8c,*	943.9 ± 25.7d,*
Heptanal	Fresh, aldehydic, fatty, green	877	32.0 ± 4.7a,*	17.2 ± 2.1b,*	15.1 ± 0.7b,*	17.2 ± 1.0b,*	44.4 ± 1.2b,*	59.6 ± 3.2a,*	63.1 ± 1.3a,*	58.5 ± 6.9a,*
Benzaldehyde	Almond	945	402.2 ± 59.0a	272.4 ± 34.3b	66.6 ± 10.4c,*	39.2 ± 7.6c,*	282.4 ± 5.6a	242.5 ± 23.3ab	216.0 ± 28.3bc,*	190.5 ± 28.5c,*
Octanal	Citrus, fat	977	9.2 ± 1.8a,*	5.2 ± 0.4b,*	2.9 ± 0.3c,*	2.1 ± 0.0c,*	24.1 ± 0.7a,*	23.5 ± 0.6a,*	20.6 ± 2.0ab,*	16.9 ± 2.9b,*
Benzeneacetaldehyde	Green, sweet, floral, hyacinth, beany	1025	1.3 ± 0.2c,*	3.2 ± 0.0b,*	4.1 ± 0.3ab,*	4.7 ± 0.7a,*	3.2 ± 0.1d,*	7.9 ± 0.5c,*	11.5 ± 0.7b,*	13.5 ± 1.2a,*
Nonanal	Waxy, fatty, citrus	1075	20.1 ± 1.6a,*	12.4 ± 1.2b,*	12.9 ± 0.7b,*	12.3 ± 1.7b,*	38.8 ± 5.1b,*	51.6 ± 4.4ab,*	54.7 ± 4.7a,*	50.4 ± 9.7ab,*
Total			2445.6 ± 101.2a,*	1126.9 ± 51.3b,*	610.8 ± 56.1c,*	495.2 ± 50.9d,*	2250.9 ± 46.7a,*	2064.8 ± 73.2b,*	1817.5 ± 43.6c,*	1522.0 ± 43.1d,*
Esters										
Acetic acid, metyl ester	Solvent-like, fruity	511	1.4 ± 0.1d,*	37.0 ± 3.8c,*	69.1 ± 5.1b,*	91.6 ± 4.6a,*	6.5 ± 1.0c,*	7.5 ± 1.3c,*	9.4 ± 0.7b,*	12.0 ± 0.8a,*
Furans										

Furan, 2-methyl-	Ethereal, acetone, chocolate	589	0.2 ± 0.0c,*	0.6 ± 0.0c,*	1.6 ± 0.2b	2.4 ± 0.3a,*	1.3 ± 0.1ab,*	1.6 ± 0.3a,*	1.5 ± 0.2ab	1.2 ± 0.1b,*
Furan, 2-ethyl-5-methyl-	Fresh, gassy, burnt	772	5.1 ± 0.7a,*	4.7 ± 0.3ab,*	4.3 ± 0.4ab,*	3.7 ± 0.3b,*	16.9 ± 0.9a,*	15.2 ± 0.4a,*	14.3 ± 0.5ab,*	12.3 ± 2.0b,*
Furan, 2-pentyl	Fruity, green, earthy, beany	963	451.7 ± 70.8a,*	350.7 ± 5.0b,*	317.7 ± 16.6b,*	278.7 ± 41.9b,*	851.6 ± 21.7b,*	1007.6 ± 57.1a,*	1041.7 ± 69.3a,*	840.9 ± 48.8b,*
Total			456.9 ± 70.8a,*	356.0 ± 5.0ab,*	323.6 ± 16.6ab,*	284.9 ± 41.9b,*	869.9 ± 21.7bc,*	1024.5 ± 57.1ab,*	1057.5 ± 69.3a,*	854.5 ± 48.9c,*
Ketones										
2,3-Butanedione	Buttery, sweet, creamy, pungent, caramellic	563	22.2 ± 2.6a	10.4 ± 0.7b,*	18.1 ± 2.9a,*	22.8 ± 3.4a,*	29.2 ± 5.4a	4.9 ± 0.6c,*	8.6 ± 0.3bc,*	11.1 ± 1.2b,*
2-Hexanone	Fruity, fungal, meaty, buttery	762	81.3 ± 7.6a,*	66.6 ± 4.0b,*	62.1 ± 4.4b,*	53.9 ± 6.9b,*	50.3 ± 9.4a,*	29.0 ± 4.1b,*	20.8 ± 3.2bc,*	14.7 ± 2.4c,*
5-Hepten-2-one, 6-methyl-	Citrus, green, musty	958	32.4 ± 3.5b	34.3 ± 6.2b	114.9 ± 11.8a,*	123.6 ± 5.5a,*	23.4 ± 3.2ab	26.9 ± 2.8a	21.6 ± 0.7bc,*	18.4 ± 1.5c,*
2-Decanone	Orange, floral, fatty, peach	1159	26.2 ± 3.3a	27.6 ± 1.1a	27.0 ± 1.5a,	23.3 ± 3.6a	33.2 ± 1.1a	29.9 ± 0.9ab	27.6 ± 1.6bc	24.3 ± 2.6c
2,3-Octanedione	Dill, asparagus, cilantro, herbal	956	12.2 ± 0.5a,*	10.5 ± 0.3b,*	7.9 ± 0.5c,*	7.2 ± 1.1c,*	31.6 ± 0.3a,*	29.3 ± 1.6ab,*	27.6 ± 1.6ab,*	25.6 ± 2.5b,*
2-Nonanone	Fresh, sweet, green, weedy	1061	54.5 ± 5.9a	53.7 ± 0.8a	57.8 ± 5.6a	49.8 ± 7.2a	62.0 ± 2.4a	54.8 ± 2.4ab	48.9 ± 2.8bc	43.6 ± 4.2c
2,3-Pentanedione	Pungent, sweet, butter, creamy	666	94.7 ± 7.0a	43.6 ± 8.2b,*	33.1 ± 4.4bc,*	24.7 ± 2.1c,*	88.9 ± 6.9a	73.3 ± 4.3b,*	59.8 ± 11.8bc,*	48.8 ± 8.1c,*
2-Heptanone	Cheese, fruity, coconut	864	235.7 ± 20.4a	226.6 ± 12.3ab	230.1 ± 10.5ab,*	205.5 ± 9.1b,*	257.3 ± 5.3a	192.4 ± 3.5b	150.8 ± 1.9c,*	119.6 ± 10.5d,*
Total			559.2 ± 24.3a	473.4 ± 16.6b,*	551.0 ± 18.1a,*	510.6 ± 15.6b,*	575.9 ± 14.5a	440.4 ± 8.1b,*	365.7 ± 13.0c,*	306.2 ± 14.7d,*
Pyrazines										
Pyrazine, 2,5-dimethyl-	Burnt, almond, roasted, nutty	896	2.3 ± 0.2b,*	3.7 ± 0.0a	3.9 ± 0.2a,*	3.9 ± 0.3a	5.0 ± 0.1a,*	3.4 ± 0.4b	5.8 ± 1.1a,*	4.7 ± 0.4ab
Sulfur compounds										
Methanethiol	Vegetable oil, beany, alliaceous, eggy, creamy	458	42.3 ± 0.9a,*	16.3 ± 1.2b	10.9 ± 0.9c	10.2 ± 1.6c,*	19.3 ± 1.1a,*	15.3 ± 0.6b	11.5 ± 1.2c	6.8 ± 0.3d,*
Carbon disulfide	Vegetable, sulfide	521	0.9 ± 0.1c,*	1.2 ± 0.1bc,*	1.4 ± 0.0ab,*	1.7 ± 0.3a,*	4.3 ± 0.8a,*	4.4 ± 0.3a,*	4.7 ± 0.8a,*	4.5 ± 0.7a,*
Thiophene	Alliaceous, garlic	643	0.0 ± 0.0a,*	0.0 ± 0.0a,*	0.0 ± 0.0a,*	0.0 ± 0.0a,*	4.4 ± 0.5a,*	3.3 ± 0.6b,*	3.2 ± 0.3b,*	2.2 ± 0.1c,*
Disulfide, dimethyl	Sulfurous, vegetable, cabbage, onion	720	2.7 ± 0.1b,*	3.5 ± 0.1a	1.8 ± 0.1c,*	1.2 ± 0.1d,*	1.9 ± 0.3c,*	2.6 ± 0.4c	4.9 ± 0.5b,*	8.7 ± 0.2a,*
Thiophene, 3-methyl-	Fatty, winey	747	0.3 ± 0.0b,*	0.4 ± 0.0a,*	0.3 ± 0.0ab,*	0.3 ± 0.0b,*	1.2 ± 0.1b,*	1.1 ± 0.2b,*	2.8 ± 0.2a,*	1.4 ± 0.1b,*
Pentanethiol	Sulfurous, fatty, roasted, meaty	797	1.0 ± 0.1a,*	0.0 ± 0.0b,*	0.0 ± 0.0b,*	0.0 ± 0.0b	2.7 ± 0.1a,*	2.0 ± 0.2b,*	1.1 ± 0.0c,*	0.0 ± 0.0d

Thiophene, 2-pentyl-	Fatty, chicken coup, cranberry	1135	2.7 ± 0.4a,*	2.5 ± 0.2a,*	2.4 ± 0.2a,*	2.3 ± 0.1a,*	6.0 ± 0.1ab,*	6.9 ± 0.3a,*	6.8 ± 0.7a,*	5.2 ± 0.8b,*
Thiophene, 2-hexyl-	Floral, fruity, gassy, green	1289	0.0 ± 0.0a,*	0.0 ± 0.0a,*	0.0 ± 0.0a,*	0.0 ± 0.0a,*	10.5 ± 0.4a,*	6.7 ± 0.5b,*	5.2 ± 0.1c,*	3.4 ± 0.2d,*
Total			50.0 ± 1.0a	23.8 ± 1.2b,*	16.8 ± 0.9c,*	15.7 ± 1.7d,*	50.3 ± 1.6a	42.3 ± 1.2b,*	40.2 ± 1.7c,*	32.3 ± 1.2d,*

PPI—Pea Protein Isolate; DWMP—Durum Wheat Meal protein. Data are expressed as a mean ± SD (n = 3). Different letters in the same row indicate significant differences according to pairwise t-test within the same category (either raw materials or TVPs). * indicates significant differences between the TVP and the corresponding raw material.

Sensory evaluation of extrudates

Upon comparing the extrudates overall, the inclusion of DWMP had a minor impact on the odor (Figure 2A) and taste profile (Figure 2B) of the TVPs. The only significant difference was an increase in roastiness (odor) due to the addition of DWMP, with PPI scoring 1.0 ± 0.5 and PPI_DWMP 60:40 scoring 2.0 ± 0.9 (p<0.05).

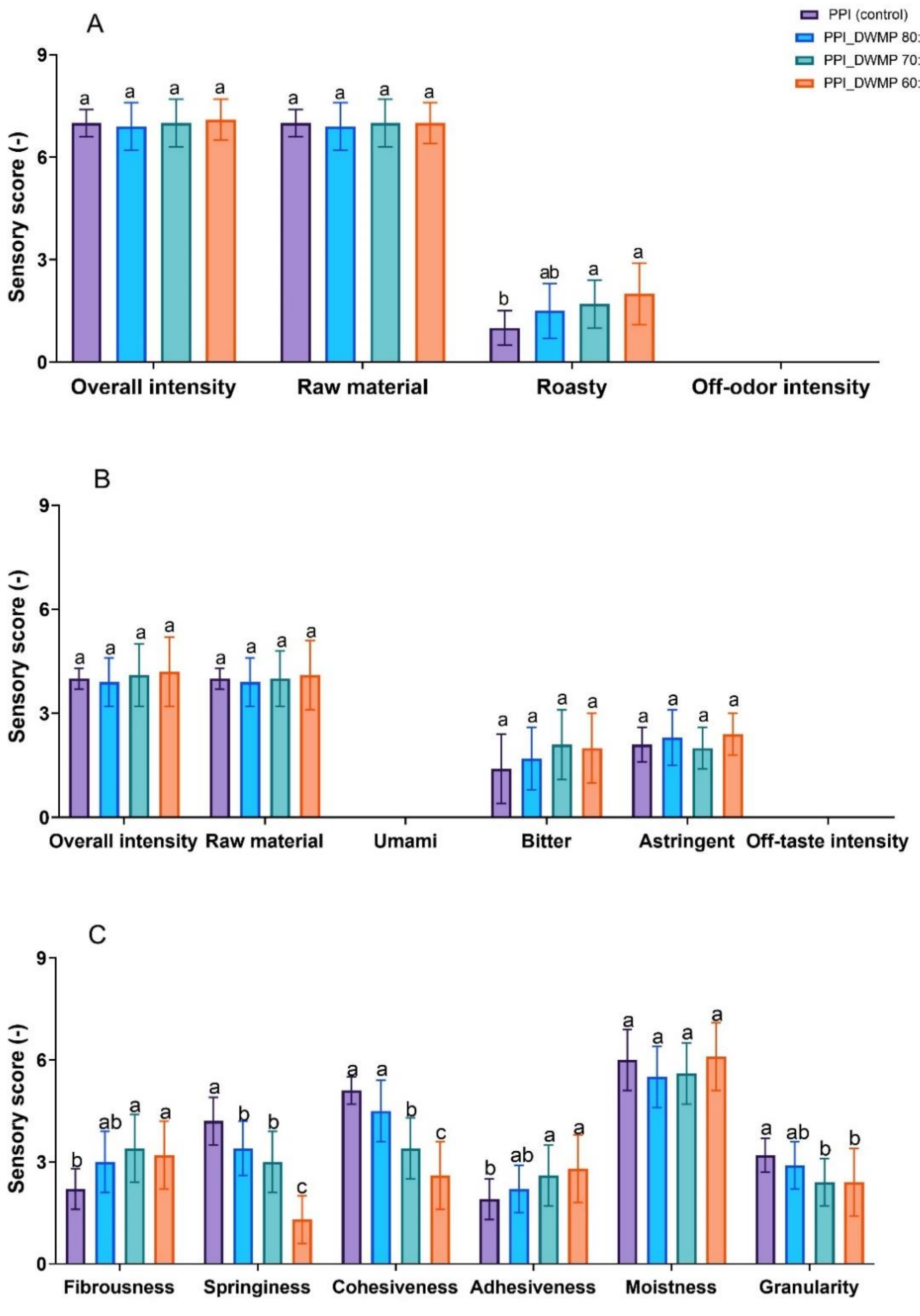


Figure 2. Results of the sensory analysis of TVPs. A: odor, B: taste, C: texture. The scale was 0–9. PPI—Pea Protein Isolate, DWMP—Durum Wheat Meal protein. Data are expressed as a mean ± SD (n = 20). Different letters within each attribute indicate significant differences according to Tukey's HSD test (p ≤ 0.05).

This increase in roastiness could be explained by the reduction of compounds responsible for green aroma in the extrudates containing DWMP, leading to a heightened perception of roastiness. However, these differences were minor, and it is likely that they would not be noticeable in the final product when flavorings are added. Interestingly, the increase in roastiness in the samples containing DWMP was not associated with off-odor intensity, as panelists did not assign any off-odor scores. Bitterness and astringency were detected at low levels, and no off-tastes were identified. Previous research indicates that improving sensory acceptability of plant-based meat alternatives relies on mitigating bitterness, astringency, and other off-flavors (Wang et al., 2022). It is also important to acknowledge that sensitivity to bitterness can vary greatly among individuals. Nevertheless, considering that pea bitterness is commonly easily detectable (Vaikma et al., 2022), the minimal bitterness of the extrudates suggests that undesirable taste attributes are unlikely to be a concern in further product development. Considering the volatile compounds discussed in the previous section, these results indicate that instrumental analysis can provide insights into changes in odor compounds in food, but it may not always directly correlate with the odor perceived by humans.

Texture perception of extrudates (Figure 2C) exhibited variation in certain attributes. Specifically, the addition of DWMP significantly reduced springiness. Significant changes in fibrousness, adhesiveness, and granularity were also observed, although the difference was only one point between the PPI and the extrudate with the highest DWMP content. Sensorial moistness was not significantly affected by the addition of DWMP contradicting the WHC results, which suggested a potential decrease of moistness. However, since the differences in WHC between the samples were small, they were not perceived by the panelists.

From these findings, it can be concluded that DWMP changed the texture of the extrudates, resulting in TVPs with reduced springiness, and cohesiveness. This is a positive outcome as efforts are made to make meat analogs more tender (Opaluwa et al., 2025). However, the final texture profile of the product may be influenced not only by the DWMP content, but also by the extrusion cooking conditions (Vu et al., 2022; Bakhsh, et al., 2021).

Conclusions

This study investigated the effect of durum wheat meal protein (DWMP) content in low-moisture texturized vegetable proteins (TVP), using a control extrudate produced purely from pea protein isolate (PPI) for comparison. The results highlight the importance of considering both sensory attributes and techno-functional properties in TVP development destined for meat alternative products. While oil absorption and protein digestibility remained unvaried, the water absorption was reduced by the addition of DWMP. However, this reduction did not cause any change in sensory perception of moistness, meaning that we may use less water to rehydrate the TVPs compared to the control. Additionally, the sensory evaluation demonstrated that no off-flavor or off-odor were detected, indicating that the use of DWMP as a food ingredient does not lead to negative sensory implications. This was a successful result, considering that sensory properties often represent a significant barrier to the utilization of by-products in food formulations, which may limit consumer expectations and acceptance.

While this study assessed the impact of DWMP on the techno-functional and sensory properties of TVPs, their performance in final product formulations was not assessed. This limitation could be addressed in future research, which may also further investigate the environmental, economic, and social sustainability implications of using DWMP as a food ingredient. Nevertheless, the results support the transition towards sustainable food systems by demonstrating how durum wheat by-products can be innovatively utilized as ingredients for TVP development, even at the 40% of incorporation. This represents a significant outcome, considering that DWMP has not previously been investigated for this utilization.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Vittoria Latrofa: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—Original Draft, Writing—Review & Editing; **Aleksei Kaleda**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing—Review & Editing; **Davide De Angelis**: Conceptualization, Data curation, Methodology, Supervision, Writing—Original Draft, Writing—Review & Editing; **Aaro Videvik**: Data curation, Formal analysis, Investigation; **Helen Vaikma**: Formal analysis, Investigation, Data curation, Writing—Original Draft; **Antonella Pasqualone**: Project administration, Resources, Writing—Review & Editing; **Carmine Summo**: Conceptualization, Project administration, Resources, Supervision, Writing—Review & Editing.

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Chapter 6. Rheological and structural properties of gelatin-free jelly candies obtained with dry-fractionated pea starch and corn starch

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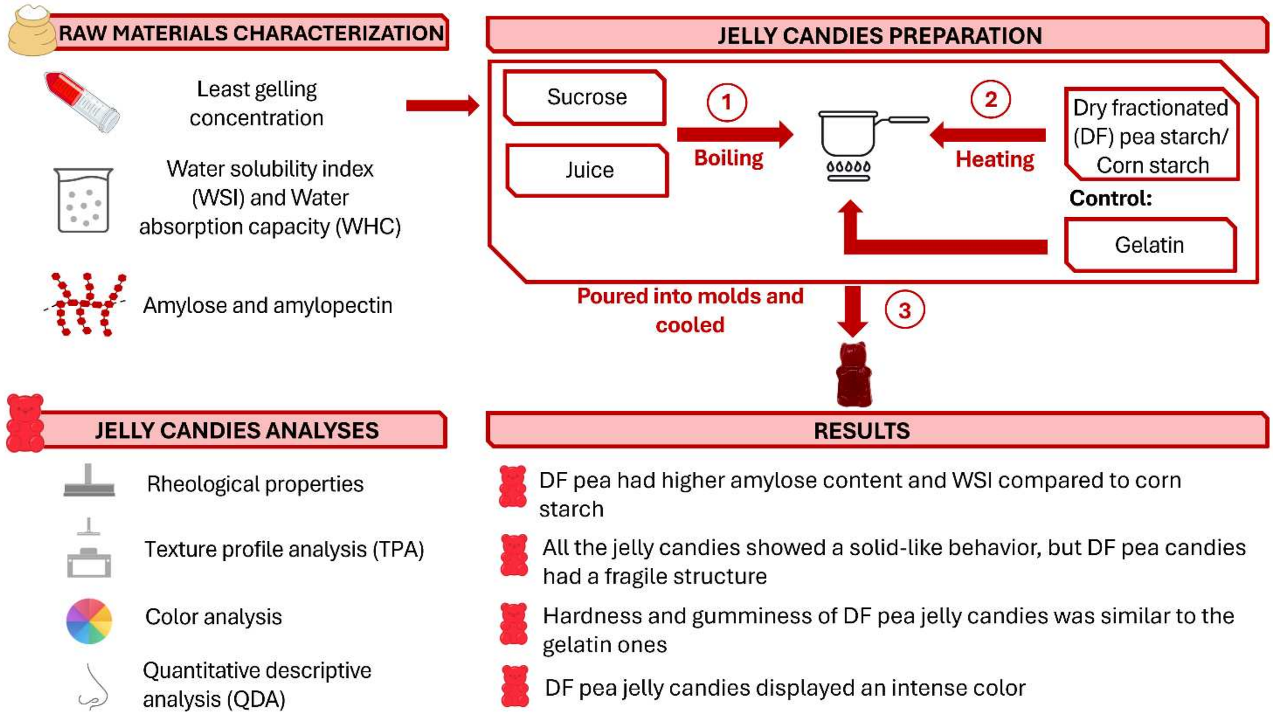
Graphical abstract



Abstract

Dry-fractionated (DF) starches are considered a co-product of protein production and remain underutilized in food applications, where isolated starches are generally preferred. This study aimed to characterize DF pea starch, compare it with conventional corn starch, and evaluate their potential as sustainable alternatives to gelatin in jelly candies. Gelatin-based jelly candies were prepared as the control (G).

DF pea starch exhibited a higher amylose content and water solubility index compared to corn starch. Least gelling concentration (LGC) was determined to be 16% for DF pea starch, 12% for corn starch, and 6% for gelatin. Based on these values, three jelly candy formulations were developed by increasing the starch content by 4% and 8% above the respective LGC. Microscale and macroscale structural behaviors were evaluated using rheological tests and texture profile analysis (TPA), respectively. All jelly candies demonstrated a solid-like structure, as evidenced by a predominance of storage modulus (G') over loss modulus (G''). DF pea-based jelly candies showed a significantly higher consistency index (1528 ± 58) than those made with corn starch (555 ± 158). However, DF pea starch candies had the lowest degree of structural recovery (mean value of 1.03%), indicating a fragile gel network, as confirmed by the texture map and TPA results. DF pea-based jelly candies exhibited a more intense color compared to the other samples. These findings suggest that, despite some structural limitations that may require further optimization, DF pea starch is promising as a plant-based gelling agent in the formulation of vegan jelly candies.

Keywords: jelly candies; starch; rheological properties; structural properties; gelling agents

Introduction

Starch can be obtained through two main methods: dry and wet fractionation. Wet fractionation, the more conventional approach, involves dehulling and wet milling followed by multiple steps of separation, washing, and purification, often using sulfur dioxide (SO_2) or acids to enhance starch-protein separation (Ren et al., 2021). By contrast, dry fractionation relies on the physical separation of micronized raw materials into a fine protein-rich fraction and a coarse starchy fraction using air classification or tribo-electric separation (Ren et al., 2021, Sweers et al., 2022; Tabtabaei et al., 2019). As a result, dry fractionation is a more sustainable alternative, requiring minimal energy and water while avoiding the use of chemicals (De Angelis et al., 2024a, Schutyser et al., 2025).

Although two distinct fractions can be obtained with dry fractionation, this process is predominantly known and investigated for the protein concentration, with the coarse starch-rich fraction often neglected and primarily used in the feed industry (Ren et al., 2021). However, there is increasing interest in exploring the potential of dry-fractionated starch for food applications. For instance, coarse fractions from durum and soft pigmented wheat have been successfully incorporated into bread formulations (Ficco et al., 2018), while pea starch fractions have been used as an alternative to wheat flour in layer and sponge cakes (Gómez et al., 2012). Starches are key functional ingredients in the food industry, commonly used as gelling agents in many food applications (Ren et al., 2021). Among others, the confectionery sector, particularly soft candies, has experienced rapid growth in recent years (Gunes et al., 2022). The candy market is projected to grow at a compound annual growth rate (CAGR) of 3.82% from 2024 to 2029 (Mordor Intelligence, 2024). Traditionally, soft confectionery products are based on starch, gelatin, or pectin as gelling agents (Gunes et al., 2022). However, the rising demand for plant-based foods has led to increased interest in vegan alternatives, particularly in the candy segment, to replace gelatin (Stępień et al., 2023).

The different types and the quantity of hydrocolloids used in preparation significantly influence the properties of the final product, especially texture (Gunes et al., 2022), which, along with color, is a key quality parameter that strongly affects consumer preferences (Burey et al., 2009; Gunes et al., 2022). Rheology is one of the primary techniques used to analyze the structure and texture of confectionery gels. It provides insights regarding the microstructure of gels (Gunes et al., 2022). Previous studies have applied rheological measurements to evaluate the kinetics and microstructure of gelatin-based confectionery gels (Wang & Hartel, 2022). Additionally, Pereira et al. (2022) explored the application of cassava starch compared to acid-thinned corn starch in gummy candy formulations, demonstrating that both the source and concentration of starch influence the product's color and texture. Li et al. (2019) investigated the differences between commercial and dry-fractionated starches, highlighting that pulse starches such as pea, lentil, and faba possess gelling properties suitable for jelly-type desserts due to their ability to form strong gels.

Given these findings, the application of pea starch obtained through dry fractionation, could represent a sustainable alternative to commercially isolated starch as a gelling agent in confectionery gels. However, the use of dry-fractionated starch in such applications, has not been previously reported. Therefore, this study aims to comprehensively investigate the rheological and textural properties of jelly candies produced using dry-fractionated pea starch. To assess its functionality, comparisons were made with commercial corn starch. Additionally, the properties of starch-based jelly candies were compared to gelatin-based formulations to evaluate the overall quality of the products.

Materials and methods

Materials

Dry-fractionated (DF) pea starch concentrate was purchased from InnovaProt s.r.l. (Gravina in Puglia, Italy), while corn starch (Cameo S.p.A., Desenzano del Garda, Italy) was purchased from a local supermarket. The starches were analyzed to determine the content of protein, starch, ash, and lipid, as described in section 2.2. Carbohydrate content was calculated as difference, respect to protein, lipid and ash. The results of the determinations indicated that DF pea starch had 16.57 ± 0.01 g/100 of protein, 1.48 ± 0.01 g/100 of lipid, 1.86 ± 0.03 g/100 of ash, 80.09 ± 0.04 g/100 of carbohydrates of which 46.16 ± 1.87 g/100g of starch, (dry matter basis)., Corn starch had 0.54 ± 0.09 g/100 of protein, 0.17 ± 0.01 g/100 of lipid, 0.26 ± 0.01 g/100 of ash, 99.04 ± 0.10 g/100 of carbohydrates of which 78.41 ± 0.13 g/100 of starch (dry matter basis). Gelatin (Cameo S.p.A., Desenzano del Garda, Italy) was purchased from a local supermarket and it had 88.3 and 0.1 g/100 of protein and lipid, respectively, according to the supplier information. Sucrose (Eridania, Bologna, Italy) and red berry juice (Zuegg, Verona, Italy) were purchased from a local retailer.

Chemical determination of starch ingredients

Protein content (N × 6.25) of DF pea starch and corn starch were determined following the method AOAC 979.09. The lipid content was determined using a Randall apparatus (SER 148 extraction system, Velp Scientifica s.r.l., Usmate Velate, Italy) according to the method AOAC 945.38F with diethyl ether as extraction solvent. Ash determination was carried out as described in the method AOAC 923.03. Total starch content was determined using total starch assay kit (Megazyme Ltd, Bray, Ireland), following the method AOAC 996.11. Briefly, 100 mg of the sample was stirred with 10 mL of sodium acetate buffer (100 mM, pH 5). Starch was then enzymatically hydrolyzed by incubating the sample with thermostable α-amylase and amyloglucosidase to obtain D-glucose molecules. These molecules were quantitatively measured through a colorimetric reaction using a glucose oxidase-peroxidase (GOPOD) reagent. The absorbance was recorded at 510 nm using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). A reagent blank was prepared by mixing 0.1 mL of distilled water with 3 mL of GOPOD reagent in duplicate. The total starch content was calculated on a dry matter basis using the following equation:

Total starch (g/100g d.m.) = $\frac{\Delta A \times F \times EV \times 0.90}{W}$

Where: ΔA is the absorbance (reaction) measured against the reagent blank, F is factor to convert 100 µg of glucose (100 µg of glucose/GOPOD absorbance value obtained for 100 µg of glucose), EV is sample extraction volume, W is the weight of the sample and 0.90 is the factor used to convert glucose into starch.

Amylose and amylopectin determination was performed using the Amylose/Amylopectin assay kit (Megazyme Ltd., Bray, Ireland), which is based on the precipitation of amylopectin using concanavalin A (Con A), following the manufacturer's instructions. The amylose concentration in the starch sample was estimated through a colorimetric reaction, with absorbance measured at 510 nm using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, USA). The amylose content was calculated as the ratio of the GOPOD absorbance of the supernatant from the Con A-precipitated sample to the absorbance of the total starch sample (Megazyme Ltd., Bray, Ireland). Amylopectin content was determined by subtracting the amylose content from 100% of the total starch.

Water absorption index, water solubility index and gelling behavior

Water absorption index (WAI) and water solubility index (WSI) of DF pea and corn starch were carried out as described in De Angelis et al. (2024b) and performed at three different temperatures: 75, 85 and 95 °C.

The least gelling concentration (LGC) of DF pea and corn starch was determined according to De Angelis et al. (2022), by increasing the concentrations of starch from 2 to 20 g (w/v). Gelatin, DF pea and corn starch were prepared as described in Section 2.4. Then, the tubes were kept at 4 °C for 2 h before determining the LGC. The LGC value was defined as the concentration below which no self-supporting gel was formed.

Jelly candies preparation

The formulations of the starch jelly candies were selected based on the results of the LGC analysis. Briefly, starting from the lowest gelling concentrations of starches, incremental additions of 4% starch were studied for jelly candy formulation. The 4% increment was chosen because preliminary rheological experiments showed no significant differences when a 2% starch increment was used. The gelatin jelly candy formulation was determined based on the LGC results while also considering the minimum gelatin content in commercial candies, which is approximately 6% (Gunes et al., 2022). The formulations are reported in Table 1, and the percentage of DF pea starch, corn starch or gelatin are expressed on the amount of juice.

Table 1. Formulation of the Jelly candies prepared with dry fractionated (DF) pea starch (P) at 16, 20, 24% (P16, P20, P24 respectively), corn starch (C) at 12, 20, 24% (C12, C16, C20 respectively) and gelatin (G) at 6%. The percentage of DF pea starch, corn starch, or gelatin is expressed on the amount of juice.

Ingredients (g)	P16	P20	P24	C12	C16	C20	G
DF pea starch	16	20	24	-	-	-	-
Corn starch	-	-	-	12	16	20	-
Gelatin	-	-	-	-	-	-	6
Juice	100	100	100	100	100	100	100
Sucrose	60	60	60	60	60	60	60

To produce the jelly candies with DF pea starch and corn starch, the juice was boiled with all the sucrose for 1 minute in a pan on an induction heater (G3 Ferrari, Rimini, Italy) at a power of 2000 W. The starch was then added, and the mixture was heated in the same conditions while stirring for 4 minutes. In the gelatin formulation, gelatin was first soaked in water (1:2 w/v) at 20 °C for 2 minutes. The juice was heated for 1 minute in a pan, after which the excess water was removed from the gelatin before adding it to the mixture. After preparation, all jelly candies were poured into molds and cooled at 4 °C for two hours before analysis. The jelly candies were produced in duplicate.

Rheological properties of jelly candies

The rheological properties of the jelly candies were measured with a rheometer (HAAKE MARS iQ Air, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a parallel plate geometry (P35/Ti-02180932). All the determinations were carried out at 0.8 mm gap between the plates and maintaining the temperature at 20 °C.

The amplitude sweep was conducted as described by O'Connell et al. (2022) with some modifications. The strain was modified from 0.01 to 1000% at a constant frequency of 1 Hz, and a linear viscoelastic region (LVR) was determined. The yield stress (kPa) and the strain (%) at the end of the LVR were plotted to generate a texture map as explained by Schreuders et al. (2022).

The oscillatory frequency sweep, the shear rate ramp test, and the structural recovery were carried out as described in Liu et al. (2021b) with some modifications. In the frequency sweep test, the frequency varied from 0.1 to 100 Hz, at a strain value of 1%, within the LVR of the jelly candies.

The shear rate ramp test was performed by increasing the shear rate from 0.1 to 100 s^{-1} at 25 °C. The data were fitted to the Ostwald–de Waele model, according to the following equation:

$\tau = K \dot{\gamma}^n$

were: τ = shear stress (Pa), K = consistency index (Pa s^n); $\dot{\gamma}$ = shear rate (s^{-1}), and n = flow behavior index (dimensionless).

The structural recovery measurement was performed following the method described in Liu et al. (2021a), with some modifications. In the first step, a shear rate of 1 s^{-1} was applied for 180 s. The sample was then subjected to a shear rate of 300 s^{-1} for 60 s, followed by a return to 1 s^{-1} for 240 s. The degree of structural recovery (DSR) was calculated as the ratio of the average viscosity (η) during the last 180 s of the third step to the average η during the last 150 s of the first step, multiplied by 100. All the rheological analyses were performed twice for each replicate (*n*=4).

Texture and color of jelly candies

The texture profile analysis (TPA) was conducted according to the method described in Kurt et al. (2022). A Z1.0 TN texture analyzer (Zwick Roell, Ulm, Germany) was used, equipped with a cylindrical probe (36 mm diameter) and a 1 kN load cell. The time between the two compressions, performed until 50% deformation was reached, was set to 2 seconds and the speed was set at 1 mm/s. The parameters evaluated were hardness (N), indicating the maximum force recorded during the first compression; springiness, calculated by the height identified during the second compression divided by the original height of the sample; gumminess (N), calculated as the hardness × cohesiveness; cohesiveness, calculated as the area of work during the second compression divided by the area of work during the first compression (De Angelis et al., 2024c).

The instrumental color determination of the jelly candies was performed using a CM-600d colorimeter (Konica Minolta, Tokyo, Japan) supported by SpectraMagic NX software (Konica Minolta, Tokyo, Japan). The color properties were determined according to the CIE Lab (International Commission on Illumination) color space. Lightness (L^*, from black to white), redness (a^*, from green to red), and yellowness (b^*, from blue to yellow) were determined. The chroma of the samples was determined as follows: $C^* = \sqrt{(a^{*2} + b^{*2})}$. The determinations of textural and the color properties of the jelly candies were performed 3 times for each replicate (*n*=6).

Sensory analysis of jelly candies

Quantitative Descriptive Analysis (QDA) of jelly candies was conducted by a panel of 9 trained members composed of 3 men and 6 women (age between 24 and 60 years). Sensory assessment was performed individually in isolated testing booths in a dedicated sensory room. The participation was voluntary, and the panellists were informed on the objectives of the study and were allowed to withdraw at any time during the analysis. Each participant provided written informed consent in which indicated that did not suffer from any food intolerances and allergies. A training session was carried out in order to get confidence with the lexicon, to determine the scale anchors and define the list and the intensity of each descriptor (De Angelis et al., 2024c). The panelists evaluated jelly candies, labeled with a three-digits alphanumeric code, through an unstructured 10 cm scale in which every panelist indicated the intensity of each descriptor. The descriptors evaluated were color (0: light red – 9: dark red), legume odor, red fruit odor, hardness, sweetness, legume flavor, red fruit flavor and astringency (all rated from 0: not perceived, to 9: very intense). The analysis was carried out in two separate sessions.

Statistical analysis

All data are expressed as mean ± standard deviation. The means of the samples were analyzed using a two-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Differences (HSD) test for multiple comparisons at a significance level of $\alpha = 0.05$, considering both the amount and type of starch (i.e., corn, DF pea). Differences between the samples and the control were evaluated using Dunnett's multiple comparisons test at a significance level of $\alpha = 0.05$. All statistical tests were performed using Minitab 19 Statistical Software (Minitab Inc., State College, PA, USA). Rheological data were processed using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA).

Results and discussion

Amylose and amylopectin content, and techno-functional properties of starch ingredients

The amylose and amylopectin content of the starch ingredients is reported in Table 2. Significant differences were observed between the two starches, with corn starch exhibiting a lower amylose content (15.48%) compared to DF pea starch (39.74%). Consequently, corn starch had the highest amylopectin content (85.52%). These findings align with previous studies on the amylose and amylopectin content of DF pea starch (Li et al., 2019; Ren et al. 2021).

Table 2. Amylose and amylopectin content, water absorption index (WAI), water solubility index (WSI) of dry fractionated (DF) pea and corn starch at different temperatures: 75°C, 85°C, 95°C.

Analysis	DF pea starch	Corn starch
Amylose %	39.74 ± 0.26a	15.48 ± 0.38b
Amylopectin %	60.26 ± 0.26b	84.52 ± 0.38a
75°C		
WAI (g/g)	3.62 ± 0.07b	5.34 ± 0.01a
WSI (%)	14.38 ± 0.25a	1.10 ± 0.13b
85°C		
WAI (g/g)	4.83 ± 0.00b	5.65 ± 0.04a
WSI (%)	10.56 ± 0.13a	0.90 ± 0.01b
95°C		
WAI (g/g)	5.56 ± 0.69a	6.36 ± 0.09a
WSI (%)	6.18 ± 0.02a	0.51 ± 0.00b

Data are expressed as a mean (n=3) ± SD. Different letters in the same row indicate significant differences according to the Tukey's HSD test ($p \leq 0.05$).

The ratio of amylose to amylopectin strongly influences the gelling ability of starch. Upon gelatinization, swollen starch granules deform, and amylose molecules act as a binder, filling gaps and facilitating the formation of a three-dimensional gel network that entraps water (Ren et al., 2021). As a result, a higher amylose content typically strengthens the gel network, by promoting retrogradation and the formation of a more rigid structure (Biduski et al. 2018; Ren et al., 2021).

The Water Absorption Index (WAI) and Water Solubility Index (WSI) at 75°C, 85°C, and 95°C are presented in Table 2. Both DF pea and corn starch showed an increase in WAI with a corresponding decrease in WSI as temperature increased. Corn starch exhibited a significantly higher WAI at 75°C and 85°C and a lower WSI across all temperatures compared to DF pea starch. Similar trends were observed by other authors on dry-fractionated quinoa starch (Solaesa et al., 2020), native and ethanol-treated maize starch (Sarifudin et al., 2020). The increase of the WAI at higher temperature reflects the swelling capacity and the hydration ability of the starch granules. In fact, native starch granules present in DF pea starch are expected to have low water absorption (Schlangen et al., 2022). Therefore, DF pea starch may require higher temperatures to gelatinize. Moreover, the decrease in WSI with increasing temperature was particularly evident in DF pea starch. The denaturation of the protein fractions still present in DF starch could explain these results, suggesting that at higher temperatures, the protein contributes to gel formation. As a result, they become less soluble in water leading to the increase in WAI (Solaesa et al., 2020).

Least Gelation Concentration (LGC) test helps determine the minimum quantity of hydrocolloid required to form a firm gel after heating. As shown in Table 3, LGC varies depending on the hydrocolloid used. In general, starch ingredients exhibited a higher LGC than gelatin, likely due to differences in gelling mechanisms and chemical composition. In starch-based ingredients, gelling is primarily driven by polysaccharides, whereas proteins play a dominant role in gelatin-based gels.

Table 3. Least gelling concentration of the ingredients.

Amount (g)	DF pea starch	Corn starch	Gelatin
2	-	-	-
4	-	-	±
6	-	-	✓
8	-	-	✓
10	-	±	✓
12	-	✓	✓
14	±	✓	✓
16	✓	✓	✓
18	✓	✓	✓
20	✓	✓	✓

- no gel; ± weak gel; ✓ firm gel (first time of appearance indicates the least gelling concentration). DF= dry fractionated.

Due to its lower starch content, DF pea starch showed a higher LGC compared to corn starch. However, gelatin exhibited the lowest LGC among all ingredients, indicating its superior gelation ability (Rawdkuen et al., 2013). It has been reported that proteins generally showed a lower LGC compared to carbohydrates (De Angelis et al., 2022). The LGC results were used to select jelly candy formulations and assess the effects of different starch ratios.

Rheological properties of jelly candies

Frequency sweep analysis

The rheological analyses were conducted to comprehensively characterize the microstructure of the three gels and to assess how different ingredients and their ratios impact gel structure.

The frequency sweep analysis evaluates the total resistance of the material as a function of linear frequency. Figure 1 depicts the results of the analysis, whereas the values of a storage modulus (G'), loss modulus (G") and tan δ at 1 Hz are presented in Table 4. All jelly candy formulations exhibited a predominance of G' over G", indicating a solid-like behavior, consistent with previous observations in soft candies (Pan et al., 2022) and confectionary gels (Wang & Hartel, 2022). Only a negligible dependence on the frequency was observed in starch-based gels. This suggests that the gel network structure was strong and stable to the modification caused by external environment (Pan et al., 2022). The starch concentration only caused changes in the absolute values, as previously seen by other authors (Ahmed & Basu, 2024). Notably, all the jelly candies containing DF pea starch showed the highest absolute values of both G' and G'' (Table 4) compared to those containing the corn starch. However, the higher tan δ value was found in the DF pea starch formulation, indicating that the inner structure was weaker compared to formulations with corn starch. This could be linked to the presence of the other components in DF pea starch, especially proteins or fibers. Nilsson et al. (2023) reported that an increase in protein content reduces tan δ during the gelation of faba bean starch-protein mixtures. Additionally, Sun et al. (2023) explained that proteins and dietary fibers tend to restrict the swelling ability of starch granules, a trend confirmed by the WAI results of DF pea starch in Table 2. The control exhibited frequency independence within the tested frequency range, as indicated by the G' parameter. A similar behavior was observed by Moraes et al. (2009) in gelatin-based gels. Moraes et al. (2009) explained that this behavior is typical of systems that form true gels, characterized by a reversible network structure. By contrast, none of the jelly candies produced with starch ingredients (i.e., corn and DF pea starch) exhibited similar behavior to the control. Therefore, future studies could be carried out to improve the structure of DF pea starch jelly candies by making them more stable to mechanical stress.

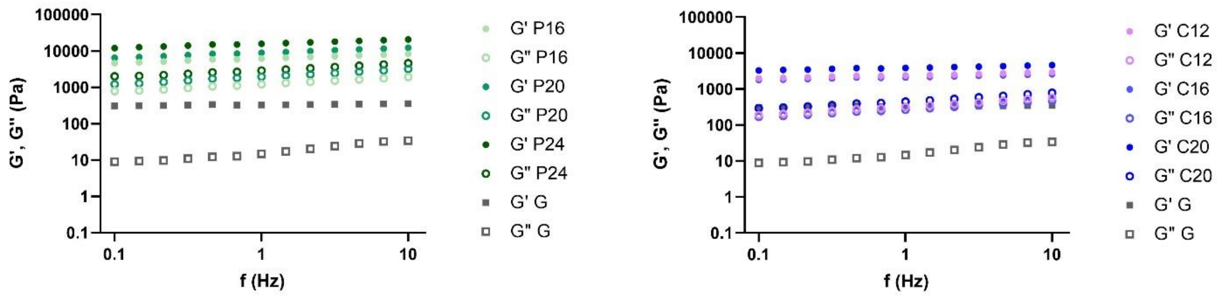


Figure 1. Mean storage modulus (G', in Pa) and loss modulus (G'', in Pa) as a function of the regular frequency (f, in Hz). Jelly candies prepared with dry fractionated pea starch (P) at 16, 20, 24% (P16, P20, P24 respectively), corn starch (C) at 12, 20, 24% (C12, C16, C20 respectively) and gelatin (G) at 6%. The bars indicate the standard deviation (*n* = 4).

Flow curves of jelly candies

The flow curves in Figure 2 illustrate the apparent viscosity (η) as a function of shear rate ($\dot{\gamma}$). Moreover, the curves were fitted using the Ostwald–de Waele model, which describes viscosity behavior. The consistency index (K) and flow behavior index (n) obtained from the model are reported in Table 4. All formulations exhibited a decrease in viscosity with increasing shear rate. This shear-thinning behavior, visible from the slope of the curves and the n value (less than 1, Table 4), is characteristic of pseudoplastic materials (Xu et al., 2021). Liu et al. (2021a) observed a similar trend in maize starch pastes, attributing the viscosity decrease to starch chain alignment in the shear direction. The highest K values were observed in samples with DF pea starch, suggesting a greater consistency compared to corn starch formulations. The higher K value in DF pea starch could be attributed to its different composition, particularly its higher amylose content. Xu et al. (2021) reported that higher amylose content correlates with increased K values. Additionally, samples at the LGC and 4% above the LGC in DF pea starch (P16 and P20, respectively) and corn starch (C12 and C16, respectively) did not show any differences in viscosity and K values. This behavior suggests that 4% increase in starch concentration does not modify the rheological characteristics of jelly candies. On the other hand, an 8% increase in starch concentration (P24 and C20) was associated with higher K values. This increase is likely due to stronger molecular associations between amylose and amylopectin at higher starch concentrations (González et al., 2021).

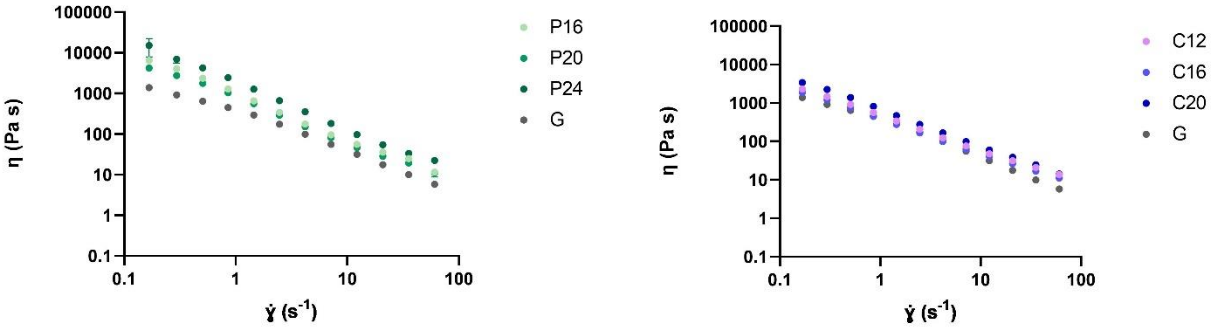


Figure 2. Mean of apparent viscosity (Pa s) as a function of the shear rate. Jelly candies prepared with dry fractionated pea starch (P) at 16, 20, 24% (P16, P20, P24 respectively), corn starch (C) at 12, 20, 24% (C12, C16, C20 respectively) and gelatin (G) at 6%. The bars indicate the standard deviation (*n* = 4).

Table 4. Rheological properties of the Jelly candies prepared with dry fractionated (DF) pea starch (P) at 16, 20, 24% (P16, P20, P24 respectively), corn starch (C) at 12, 20, 24% (C12, C16, C20 respectively) and gelatin (G) at 6%. 4% (P20; C16) and 8% (P24; C20) indicate the concentration of ingredient above the respective LGC.

	Frequency sweep			Shear rate		Structural recovery
Sample	G' (Pa)	G" (Pa)	tan δ	K (Pa s^n)	n	DSR (%)
P16	6156 ± 457c	1206 ± 68c	0.20 ± 0.00b	1506 ± 120b	0.24 ± 0.01a	0.40 ± 0.02d
P20	8847 ± 451b	1953 ± 102b	0.22 ± 0.00c	959 ± 70b	0.20 ± 0.01b	2.27 ± 1.11d
P24	15725 ± 543a	2864 ± 120a	0.18 ± 0.00a	2121 ± 54a	0.12 ± 0.01de	0.42 ± 0.12d
C12	2374 ± 108e	317 ± 20de	0.13 ± 0.00e	521 ± 42e	0.14 ± 0.01d	70.67 ± 0.72a
C16	2107 ± 52e	268 ± 8e	0.13 ± 0.00e	417 ± 19e	0.18 ± 0.01c	43.52 ± 0.89b
C20	3849 ± 109d	448 ± 18d	0.12 ± 0.00d	727 ± 10d	0.10 ± 0.01e	15.94 ± 1.64c
p-value type of starch × amount	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
DF pea starch	10242 ± 51a	2007 ± 27a	0.20 ± 0.00a	1528 ± 581a	0.19 ± 0.06a	1.03 ± 1.09b
Corn starch	2776 ± 33b	344 ± 6b	0.13 ± 0.00b	555 ± 158b	0.14 ± 0.04b	43.38 ± 23.72a
p-value type of starch	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
LGC	4265 ± 0c	761 ± 0c	0.16 ± 0.00b	1013 ± 696b	0.19 ± 0.07b	35.53 ± 38.49a
4%	5477 ± 0b	1110 ± 0b	0.17 ± 0.00a	688 ± 383c	0.19 ± 0.02a	22.89 ± 22.61b

8%	9787 ± 0a	1656 ± 0a	0.15 ± 0.00c	1424 ± 986a	0.11 ± 0.01a	8.18 ± 8.56c
p-value amount	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
G	349 ± 10	15 ± 0	0.04 ± 0.00	389367 ± 13249	0.34 ± 0.03	31.15 ± 0.88

Data are expressed as a mean (*n*=3) ± SD. Different letters indicate significant differences according to the Tukey's HSD test ($p\leq 0.05$). * means that the value is similar to the control (G) according to the Dunnett's test ($p>0.05$). G'=storage modulus; G''= loss modulus; K=consistency index; *n*=flow behavior; degree of structural recovery (DSR).

In-shear structure recovery

In-shear structural recovery of the jelly candies is shown in Figure 3, where viscosity (η) is plotted against time (s), and the degree of structural recovery (DSR) is reported in Table 4. When high shear stress was applied (second phase in Figure 3) to the samples, viscosity decreased sharply, indicating a rapid response to strain changes (Cheng et al., 2023). However, this effect was particularly pronounced in gels containing corn starch or gelatin, whereas in gels containing DF pea starch, the viscosity decline was more gradual. This phenomenon suggested lower flexibility and reduced macromolecular orientation under shear stress (Cheng et al., 2023). The ability of the gels to recover their initial viscosity, after the high shear conditions, varied depending on the type of starch used. Notably, all samples containing DF pea starch exhibited poor structural recovery, as indicated by their lower DSR values compared to formulations containing corn starch and gelatin (Table 4). Typically, materials with a high recovery percentage have DSR values exceeding 70% (Tian et al., 2024). Only C12 formulation met this criterion (70.67%). Liu et al. (2021a) highlighted that DSR is indicative of amylose recovery after shear stress. The superior structural recovery of corn starch formulations may be attributed to stronger amylose-amylopectin interactions (Liu et al., 2021a). Pang et al. (2022) suggested that during gel formation, starch pastes develop an elastic network that resists deformation and recovers after disruption. It can be hypothesized that the presence of proteins in DF pea starch gels may have inhibited the elastic recovery response after the high-shear step.

This analysis provides insight regarding the ability of jelly candies to restore their original structure after exposure to high-shear conditions, which are common during food processing operations such as pumping, mixing, and sensory chewing (Gunasekaran et al., 2000; Gunes et al., 2022). However, further verification using Large Amplitude Oscillatory Shear (LAOS) tests could be assessed in future studies, as these tests provide detailed insights into rapid and large deformations that simulate real industrial processes (Gunasekaran et al., 2000). It is important to note that no significant similarities were found in DSR of the experimental jelly candies. Nevertheless, since the lowest DSR value was found in the DF pea starch jelly candies, future research could focus on improving the formulations containing DF pea starch, in order to enhance their response to strain changes, making them more suitable to substitute gelatin.

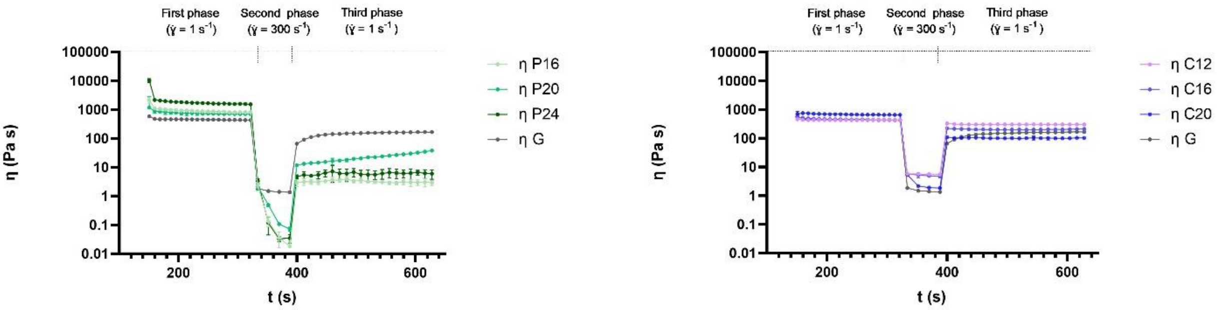


Figure 3. Mean of in-shear structural recovery (Pa s). Jelly candies prepared with dry fractionated pea starch (P) 16, 20, 24% (P16, P20, P24 respectively), corn starch (C) at 12, 20, 24% (C12, C16, C20 respectively) and gelatin (G) at 6%. The bars indicate the standard deviation (*n* = 4).

Texture map and overall rheological properties of jelly candies

The texture map in Figure 4 summarizes the rheological data in a two-dimensional plot, representing stress and strain at the end of the LVE regime (Schreuders et al., 2022). The jelly candies P20 and P16, produced with dry-fractionated DF pea starch, tended toward the mushy quadrant, whereas those containing corn starch (C12, C16, C20) were characterized by high yield stress and low shear strain, which define them as rubbery. This corroborates the results of the structural recovery, demonstrating high elasticity of the gels based on corn starch. Meanwhile, the control G (gelatin-based jelly candy) tended toward the tough quadrant, characterized by high yield stress and high shear strain. The differences in gel properties among samples can be attributed to the type of hydrocolloid used, as the gelling agent significantly influences gel structure (Gunes et al., 2022). The texture map results align with the rheological findings, particularly the tan δ values, which confirmed that DF pea starch jelly candies had a weaker internal structure, as evidenced by their mushy texture. Samples P24 and C20 were the closest to the control (G). Therefore, it can be hypothesized that blending the two starches (i.e., DF pea and corn starch) could be the next step in order to develop a product with similar structural characteristics to the jelly candies made with gelatin. However, it is important to note that texture is influenced by interactions at both macroscopic and microscopic levels. Consequently, texture mapping alone is insufficient for evaluating the structure of the final product. Texture Profile Analysis (TPA) should be conducted to obtain a comprehensive assessment. Dahl et al. (2024) suggested that both rheology and TPA should be used in soft matter studies to evaluate product structure. While TPA provides macroscopic, empirical data correlated with mouthfeel sensations, rheology provides microscale insights into gel network structure (Dahl et al., 2024). Therefore, in soft matter research, both techniques should be combined for a thorough analysis of product structure.

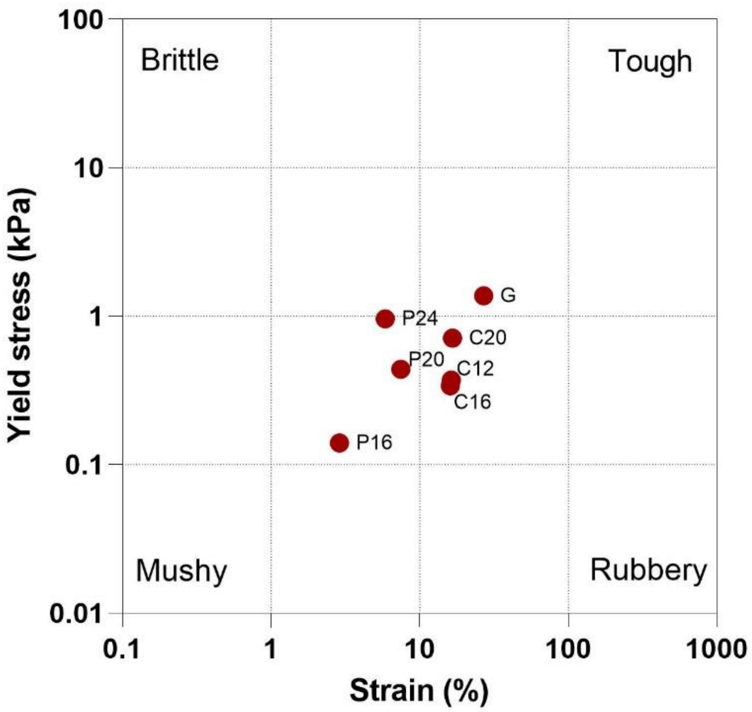


Figure 4. Texture map at the end of the linear viscoelastic region (LVR) of the jelly candies prepared with dry fractionated pea starch (P) at 16, 20, 24% (P16, P20, P24 respectively), corn starch (C) at 12, 20, 24% (C12, C16, C20 respectively) and gelatin (G) at 6%.

Texture properties

The textural properties of the jelly candies assessed through a compression test, including hardness (N), springiness, gumminess (N), and cohesiveness, are presented in Table 5. The hardness of the jelly candies varied depending on both the type and amount of hydrocolloid in the formulation. The jelly candies made with corn starch exhibited the lowest hardness values, particularly in the C12 formulation. The only sample that showed a hardness value similar to control G was P16, which is noteworthy since hardness is a critical factor influencing consumer preference (Gunes et al., 2022). Starch is well known for its ability to increase the hardness of soft candies (Gunes et al., 2022). Therefore, achieving a texture similar to gelatin could be advantageous if starch is used as a substitute for gelatin. In general, hardness increased with higher starch concentrations. Similar hardness values were observed by Kurt et al. (2022) in gummy candies made with gelatin. Springiness values were higher in corn starch jelly candies compared to DF pea starch-based ones, corroborating the results discussed with the texture map. However, both starch-based candies exhibited lower springiness compared to the control. Similar ranges of springiness were reported by Pereira et al. (2022) in gummy candies made with acid-thinned cassava starch and by de Avelar & Efraim (2020) in jelly candies. Gumminess was not affected by the type of starch but was influenced by its concentration. As the starchy ingredient content increased, gumminess also increased. Compared to previous studies (Pereira et al., 2022; de Avelar & Efraim, 2020), the gumminess values observed in this study were lower. However, Gunes et al. (2022) pointed out that low chewiness could be beneficial for consumers, as it requires less energy for chewing. Among all samples, only P24 exhibited a gumminess value similar to control G. Jelly candies made with corn starch exhibited higher cohesiveness than those made with DF pea starch. Additionally, cohesiveness decreased with increasing starch content only in corn starch gels. This parameter explains the resistance to mechanical deformation, indicating whether the gel structure remains intact after parallel compression (Dahl et al., 2024). The lower cohesiveness in DF pea starch-based jelly candies suggests a weaker and more fragile structure compared to corn starch formulations, a finding supported by the rheological data (Table 4) and by the texture map. Overall, the analysis of texture could give information about the sensory properties of jelly candies and can be complemented with rheological properties of the gel to further explain gel behavior under shear stress. Both methods highlighted significant textural differences, which were primarily attributed to differences in chemical composition. Overall, the similarities found between DF pea-based jelly candies and the control suggest that DF pea starch has the potential to substitute gelatin in vegan jelly candies, although further optimization of the overall structure could be achieved in future studies.

Color analysis

The visual appearance of the jelly candies can be examined in Figure 5, while the results of instrumental color evaluation of the jelly candies including lightness (L^*), redness (a^*), and yellowness (b^*), and chroma (C^*) are reported in Table 5. DF pea starch jelly candies exhibited the lowest L^* compared to the corn starch-based jelly candies. Redness (a^*) was the dominant color coordinate, while b^* values remained low, particularly close to zero in corn starch-based jelly candies, suggesting a more neutral color tone.

Similar color indexes values were reported by Delgado & Bañón (2018) in starch-based gummy candies, however the same authors used coloring agent to achieve this type of color. This result could be positive in terms of reducing synthetic food colorants commonly used in soft confectionery products (Gunes et al., 2022). The color indices were significantly affected by starch content, but only at the highest concentration. Interestingly, gelatin-based jelly candies appeared less reddish than starch-based formulations, a phenomenon previously reported by Delgado & Bañón (2018) in starch and inulin-based gummy jellies. These authors explained that the color differences were due to the inherent optical properties of the hydrocolloid used. Since gelatin is colorless in water, the red hue of the gelatin-based jelly candies depended entirely on the fruit juice color (Delgado & Bañón, 2018).

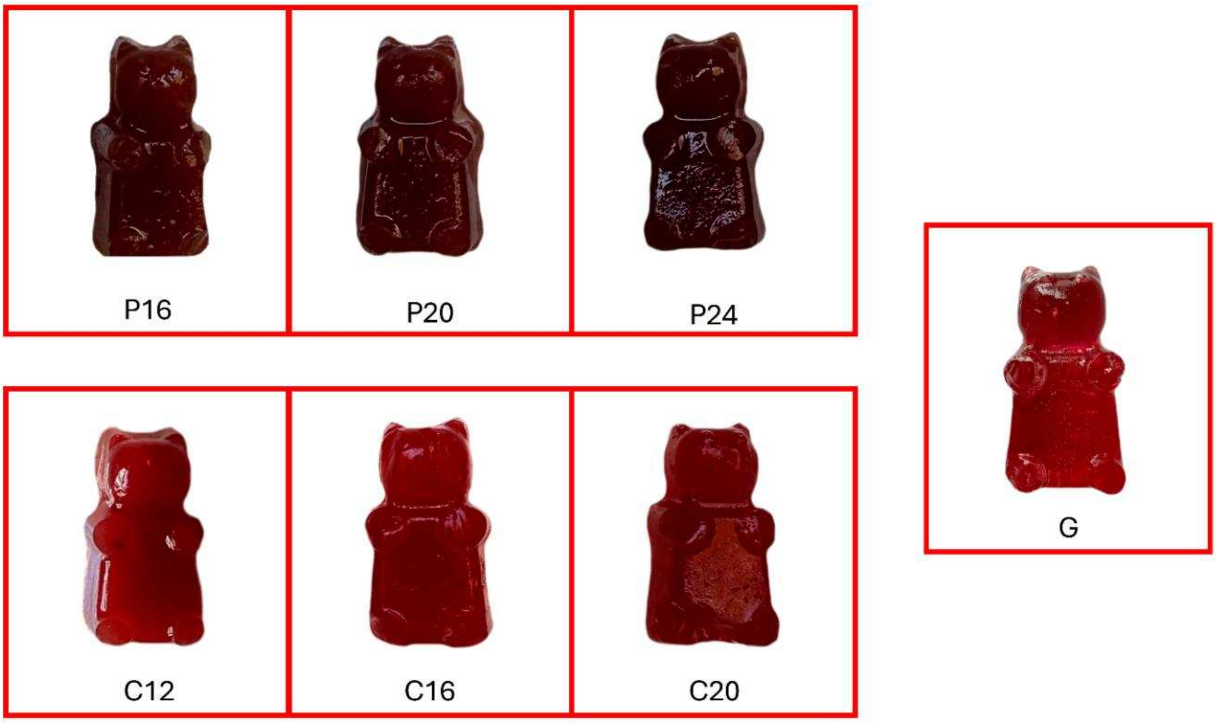


Figure 5. Visual appearance of the jelly candies prepared with dry fractionated pea starch (P) at 16, 20, 24% (P16, P20, P24 respectively), corn starch (C) at 12, 20, 24% (C12, C16, C20 respectively) and gelatin (G) at 6%.

The most pronounced differences were observed in chroma (*C**) values, which were highest in DF pea starch-based jelly candies, indicating a more intense color compared to samples containing corn starch or gelatin. The *C** values of corn starch-based jelly candies and the control reflect their more transparent appearance, aligning with findings by Moghaddas Kia et al. (2020). The differences in color appearance between corn starch and DF pea starch jelly candies could be attributed to difference in the degree of purification of their raw materials. DF pea starch has a naturally greener hue compared to corn starch because it contains other micro and macromolecules, such as minerals and proteins, which may contribute to the observed color variations. In confectionery gels, color plays a crucial role in consumer acceptance, as it is often associated with flavor perception (Altınok et al., 2020). Therefore, further optimization could involve blending DF pea starch with corn starch to optimize color as well as other structural properties.

Table 5. Texture and color of the Jelly candies prepared with dry fractionated (DF) pea starch (P) at 16, 20, 24% (P16, P20, P24 respectively), corn starch (C) at 12, 20, 24% (C12, C16, C20 respectively) and gelatin (G) at 6%. 4% (P20; C16) and 8% (P24; C20) indicate the concentration of ingredients above the respective LGC.

Sample	Hardness (N)	Springiness	Gumminess (N)	Cohesiveness	*L**	*a**	*b**	*C**
P16	1.24 ± 0.04d*	0.46 ± 0.04d	0.29 ± 0.02c	0.23 ± 0.02d	10.62 ± 0.32c	11.32 ± 0.55c	3.57 ± 0.29c	43.06 ± 0.45b
P20	1.56 ± 0.05c	0.50 ± 0.02cd	0.34 ± 0.03bc	0.22 ± 0.02d	9.84 ± 0.40d	11.19 ± 0.21c	4.08 ± 0.16b	45.66 ± 2.64b
P24	3.10 ± 0.08a	0.56 ± 0.04bc	0.67 ± 0.07a*	0.22 ± 0.02d	9.05 ± 0.02e	14.35 ± 0.40a	5.09 ± 0.36a	73.00 ± 6.14a
C12	0.77 ± 0.02f	0.64 ± 0.03a	0.33 ± 0.03bc	0.43 ± 0.03a	13.74 ± 0.05a*	13.29 ± 0.63ab	0.24 ± 0.01d	3.15 ± 0.31c*
C16	1.05 ± 0.07e	0.60 ± 0.05ab	0.39 ± 0.05b	0.37 ± 0.03b	12.52 ± 0.27b	12.87 ± 0.78b	0.36 ± 0.02d*	4.67 ± 0.48c*
C20	2.00 ± 0.18b	0.50 ± 0.02cd	0.60 ± 0.08a	0.30 ± 0.02c	10.52 ± 0.28cd	13.11 ± 0.33b	-0.32 ± 0.02e	4.19 ± 0.17c*
p-value type of starch × amount	*<0.001*	*<0.001*	*0.008*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
DF pea starch	1.97 ± 0.84a	0.51 ± 0.05b	0.43 ± 0.18a	0.22 ± 0.02b	9.84 ± 0.73b	12.29 ± 1.57b	4.24 ± 0.71a	53.91 ± 14.57a
Corn starch	1.28 ± 0.55b	0.58 ± 0.07a	0.44 ± 0.13a	0.37 ± 0.06a	12.26 ± 1.42a	13.09 ± 0.58a	0.09 ± 0.31b	4.00 ± 0.73b
p-value type of starch	*<0.001*	*<0.001*	*0.606*	*<0.001*	*<0.001*	*0.001*	*<0.001*	*<0.001*
LGC	1.01 ± 0.24c	0.55 ± 0.1a	0.31 ± 0.03c	0.33 ± 0.11a	12.18 ± 1.72a	12.31 ± 1.19b	1.90 ± 1.79b	23.11 ± 21.34b
4%	1.31 ± 0.27b	0.55 ± 0.07a	0.36 ± 0.05b	0.29 ± 0.08b	11.18 ± 1.5b	12.03 ± 1.04b	2.22 ± 1.99a	25.17 ± 21.98b
8%	2.55 ± 0.59a	0.53 ± 0.04a	0.63 ± 0.08a	0.26 ± 0.05c	9.79 ± 0.82c	13.73 ± 0.75a	2.38 ± 2.90a	38.60 ± 37.00a
p-value amount	*<0.001*	*0.271*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.001*	*<0.001*
G	1.25 ± 0.04	0.78 ± 0.01	0.72 ± 0.03	0.58 ± 0.03	13.66 ± 0.16	4.46 ± 0.23	0.64 ± 0.03	2.87 ± 0.28

Data are expressed as a mean (n=3) ± SD. Different letters for the same parameter mean significant differences according to the Tukey's HSD test (p≤0.05).

* means that the value is similar to the control (G) according to Dunnett's test (p>0.05).

Sensory properties

Efforts to redesign foods for sustainability must consider both instrumental measurements and sensory evaluations to ensure that product structure and taste align with consumer expectations (Gunes et al., 2022). Sensory properties of jelly candies are presented in Figure 6. Differences in sensory descriptors indicate that the use of the two starches resulted in distinct sensory profiles. Jelly candies produced with DF pea starch exhibited a more intense color compared to those made with corn starch, which were similar in appearance to the control sample (G). These visual differences are consistent with the instrumental color analysis, particularly the chroma index (Section 3.4).

Despite the presence of DF starch, the legume odor score remained below 2, indicating a very low perception in the candies. This odor, associated with volatile compounds known to produce a green, legume-like aroma in foods containing pulses (Roland et al., 2017), is typically observed in dry-fractionated ingredients (De Angelis et al., 2024a). The perception of red fruit odor decreased with increasing levels of both starches in the formulation, likely due to interactions between starch and aroma compounds of the juice (Pereira et al., 2022).

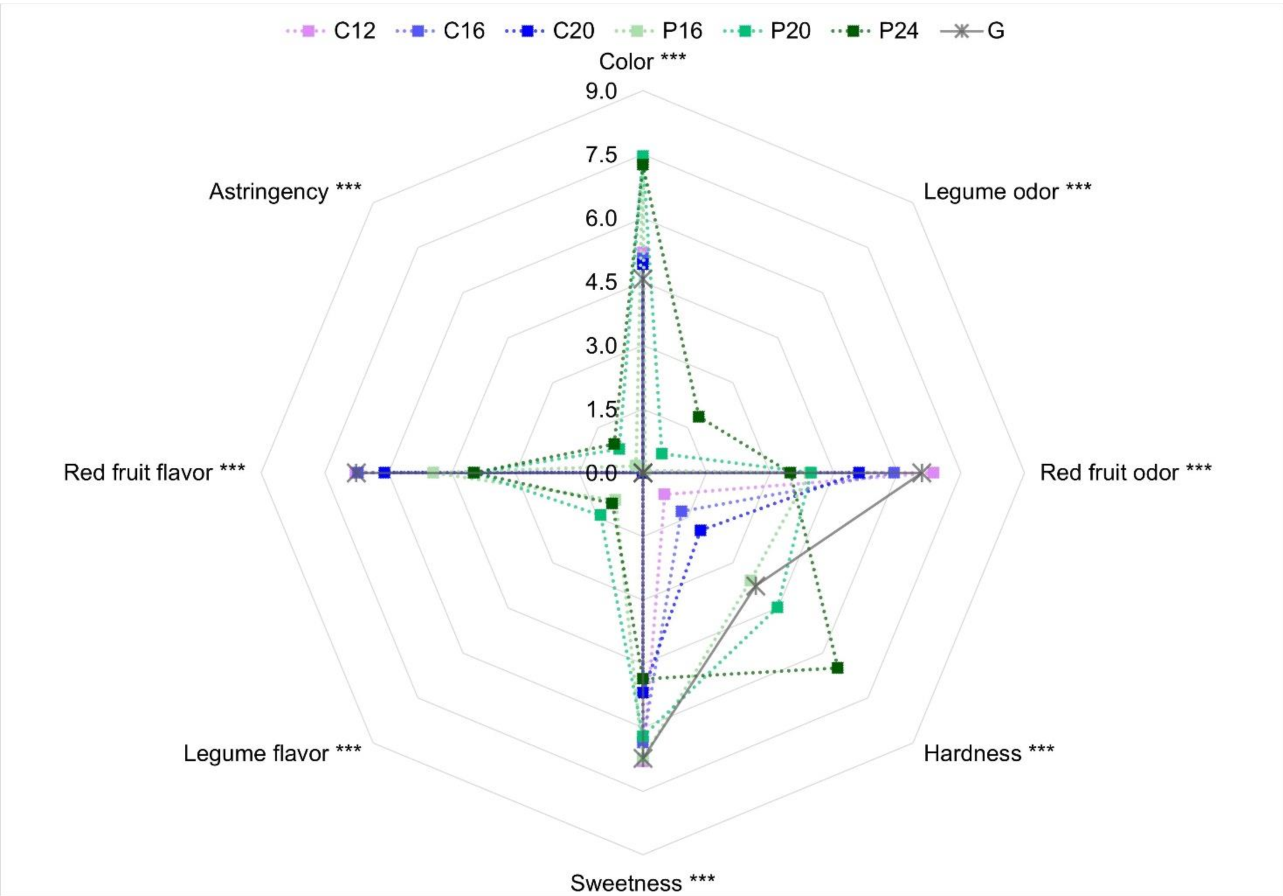


Figure 6. Sensory properties of the jelly candies prepared with dry fractionated pea starch (P) at 16, 20, 24% (P16, P20, P24 respectively), corn starch (C) at 12, 20, 24% (C12, C16, C20 respectively) and gelatin (G) at 6%.

Sensory analysis of hardness showed higher values in samples containing DF pea starch compared to those made with corn starch, aligning with instrumental hardness measurements. Comparable sensory hardness was reported in previous studies on starch-based soft candies (Jangchud et al., 2013; Mutlu et al., 2018). Sweetness was perceived as more intense in the jelly candies produced with the lowest starch concentration. This may be related to their lower hardness, which facilitates rapid disintegration in the mouth and thus enhances sweetness perception (Gunes et al., 2022; Delgado & Bañón, 2018; Pizzoni et al., 2015). Interestingly, one approach to lowering calorie content and sugar intake is enhancing sweetness perception (Gunes et al., 2022). Similar sweetness intensities were observed in other starch-based candies (Jangchud et al., 2013). The legume flavor intensity was generally low in the DF pea starch-based candies, while the predominant flavor note was attributed to the red fruit juice. This flavor was perceived as more intense in corn starch-based samples than in DF pea starch-based samples. It can be hypothesized that, although the legume flavor was mild, it may have partially masked the red fruit notes, leading to a lower perceived intensity.

Astringency was observed only in DF pea starch-based samples, with P16 showing scores near 0 like the corn starch- and gelatin-based candies. Astringency is an unpleasant oral sensation usually given by legumes (Amarowicz et al., 2004; Wang et al., 2022), though it is also influenced by individual sensitivity and salivary flow mechanisms (Wang et al., 2022).

Future perspectives

Confectionery gels, such as jelly candies, are high-sugar systems that exhibit different textural attributes depending on the hydrocolloid used (Burey et al., 2009). From an industrial perspective, the incorporation of DF pea starch in jelly candy production, instead of animal-derived gelatin, represents a significant opportunity for the valorization of this plant-based ingredient, especially given its status as a less valued co-product of protein fractionation. Dry-fractionation technology is recognized for its low energy requirements (Schutyser et al., 2025), contributing to the shift to greener and more environmentally friendly food systems (Pulivarthi et al., 2023, Schutyser et al., 2025). Its utilization could reduce reliance on animal-derived gelling agents such as gelatin, supporting the development of vegan-friendly and clean-label products, both growing trends in the confectionery market. The incorporation of DF pea starch into food products can improve the economic and environmental feasibility of the overall protein extraction process (Rajpurohit and Li, 2023), especially in comparison with starches produced through the traditional, water-consuming, wet isolation methods.

However, scaling the industrial application of DF starch in jelly candies requires further development, particularly with regard to the improvement of microstructural stability and shelf-life evaluation. Gunes et al. (2022) highlighted that weak gel structures in soft confectionery products may suffer deformation during handling, storage, and distribution. Consequently, future research should focus on optimizing the gel network, for instance by combining DF starch with other hydrocolloids such as pectin or agar, which are commonly used in confectionery formulations to reinforce texture and stability (Gunes et al., 2022). In addition, investigating the impact of processing parameters such as cooking temperature, pH, and cooling rate on the gelation behavior of DF starch could lead to tailored textural properties suited for different confectionery formats (e.g., toffee, gummy and chewy candies). Further studies are also needed to assess how DF pea starch-based candies maintain texture, color and moisture content over time, which is relevant to industry and commercial viability.

Conclusions

The application of starch as a hydrocolloid is well established in jelly candy formulation. In this study, starch obtained through isolation technology was compared to starch derived via dry fractionation technology, with the aim of understanding their effects on the structure of jelly candies from both micro- and macroscale perspectives. Rheological data revealed that jelly candies produced with DF pea starch exhibited a weaker microstructure compared to those made with corn starch and gelatin. Furthermore, structural recovery analysis revealed a very low degree of structural recovery for DF pea starch candies, indicating that, after exposure to high-shear conditions, the gels were generally unable to recover their original structure and confirmed the existence of a fragile gel network. This result was further confirmed by the texture map. Moreover, the texture profile analysis showed that certain parameters of jelly candies formulated with DF pea starch, such as hardness and gumminess were comparable to those of the control. Therefore, despite the weaker microstructure, DF pea starch can still perform effectively in terms of textural quality and could potentially be used as a gelling agent to replace gelatin in vegan jelly candies. By combining the findings from the rheological and texture analyses, it is evident that further formulation optimization could improve the overall structural integrity of the products. One promising strategy could involve blending the two types of starches.

In terms of visual properties, jelly candies made with corn starch were more transparent and exhibited a less intense color compared to those made with DF pea starch. These results suggest that DF starch can be effectively applied as a gelling ingredient in jelly candy production. This finding is particularly relevant considering that DF starch is typically a co-product of protein extraction, often relegated to animal feed. Therefore, its use in jelly candies could represent a cost-effective and sustainable approach to valorizing this ingredient.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Vittoria Latrofa: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review and editing; **Davide De Angelis**: Conceptualization, Data curation, Investigation, Supervision, Writing – review and editing; **Giacomo Squeo**: Supervision, Writing – review and editing; **Francesco Caponio**: Writing – review and editing; **Antonella Pasqualone**: Supervision, Writing – review and editing; **Carmine Summo (†)**: Conceptualization, Formal analysis, Funding Acquisition; Writing – review and editing.

DECLARATION OF CONFLICT OF INTEREST

The authors report no conflict of interest

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Chapter 7. Conclusions and future perspectives

This dissertation thesis was focused on the characterization of plant-based ingredients obtained from different extraction technologies, understanding their role in food production, and investigating their possible application in innovative foods. Dry fractionation is a sustainable technology that represents an alternative to the alkaline extraction and isoelectric precipitation (or wet fractionation) commonly used for protein and starch extraction, due to the high purity of the final ingredient. While it typically results in lower purity of the ingredients, dry fractionation preserves the native structure of proteins better, maintaining important technological and functional properties. Moreover, the other chemical components in the ingredients besides protein and starch, such as fibers, could be advantageous from a nutritional point of view. These characteristics can be highly valuable in various food applications, in order to fortify foods. In view of pursuing research on sustainable ingredients from legumes, cereal and by-products, dry fractionation represented a technology in line with the mission M2C1 of PNRR “Sustainable agriculture and circular economy” and the 12 Goal established by the 2030 United Nation Agenda “Responsible consumption and production”. The aims of this PhD project were to i) characterize plant-based ingredients and compare them with wet-fractionated ones, understanding the mechanisms responsible for different techno-functional properties and ii) apply them in food products. Indeed, the application of dry-fractionated ingredients in the food sector is still limited, and this PhD thesis aims to serve as a starting point in encouraging its application in the food industries.

In order to gain a comprehensive understanding of the potential applications of dry-fractionated proteins, information about their techno-functional properties were collected. This approach was necessary to acquire detailed knowledge of those plant-based ingredients. The results highlight that dry-fractionated proteins offer promising potential in food development, due to high solubility, gelling capacity, foaming ability and stability, compared to wet-fractionated proteins. Moreover, this technology could be applied to many different by-products from industries to obtain high value raw materials. However, challenges remain, linked to the antinutritional factors and distinct legume-related sensory profiles, which need to be explored in future research.

Dry-fractionated proteins were then compared with the wet extracted ones, because the latter are widely used in the food sector, but they are much more energy and resource consuming. This research was important to demonstrate that dry-fractionated proteins have potential as plant-based ingredient for food product development, and to assess the differences due to the protein extraction method. The latter strongly influenced protein properties. Wet extraction technology allows to modulate the protein structure, offering a wide range of ingredients with different techno-functional properties. By contrast, dry fractionation preserves the native structure, resulting in different technological and functional properties, such as gelling, foaming stability and emulsion capacity. Furthermore, dry-fractionated proteins also maintain their chemical structural features like disulfide bonds, making them more versatile for food applications. However, the comparisons presented in this study were subject to certain limitations related to differences in the composition of the raw materials. Variations in key components such as protein, carbohydrate, and fiber content certainly influenced the functional behavior of the ingredients, making it difficult to directly attribute observed differences only to the extraction methods. As a result, the functionality of raw materials may reflect the compositional differences, in some cases, more than the extraction method. Future studies could overcome these limitations by selecting ingredients with comparable compositions and then evaluating the impact of different extraction methods, in order to have a more accurate assessment of the effect of the extraction process on the functional properties of the ingredients.

Based on these results, it was possible to design food products, also using dry-fractionated by-products from the durum wheat supply chain. In this context, innovative applications of dry-fractionated ingredients were investigated, for example using them as egg replacers due to their foaming ability, as a source of protein and fibers in snack bars, to produce meat analogs due to their fibrous texture after extrusion-cooking and to act as gelling agents in jelly candies.

Egg replacement is gaining global interest especially in the plant-based bakery products sector. The results from this PhD thesis could be useful in this perspective. Indeed, using an aqueous dispersion of mung bean, lentil and chickpea dry-fractionated protein concentrate, it was possible to mimic egg in sponge cake. Three different concentrations at 20, 30, and 40% (water and dry-fractionated protein w/w) were tested, proving that the different concentration significantly influenced rheological behavior during baking. Compared to the egg, the dry-fractionated proteins increased the elastic-like behavior of the batters, as was seen from the rheological tests. Surprisingly, all the sponge cakes containing the egg replacer displayed crumb structures similar to the sponge cake produced with eggs, regardless of protein type or concentration. Since sensory aspects of legume-based products represent a drawback in their application, sensory properties were evaluated, proving that chickpea and lentil proteins imparted less legume flavor than mung bean protein. Further perspectives could be aimed at nutritional aspects, such as amino acid composition and digestibility, and to evaluate consumer acceptability of sponge cakes produced with the egg replacer.

Healthy ready-to-eat snacks are demanded by modern consumers. Therefore, in this PhD thesis the application of dry-fractionated durum wheat cake was explored. Durum wheat cake is a by-product of durum wheat supply chain that is obtained after the defatting process and the dry fractionation. The protein rich material is also rich in fibers and could be useful to improve the nutritional properties of foods. The study was conducted using experimental designs for mixtures approach to decide the formulation of a clean label snack bar. The results demonstrated that when used at levels below 10%, dry-fractionated durum wheat cake improves the snack bar nutritional profile, enhancing the protein and fiber content while reducing fat, without compromising texture or taste. Indeed, the optimized formulation could have been labeled with the nutrition claims, “low fat” and “source of fiber” in accordance with EC Regulation 1924/2006. Additionally, as it is made entirely from plant-based ingredients and contains no additives, the snack bar could have been considered a clean-label product, aligning with the preferences of both vegetarian and vegan consumers. As a future step, the snack bar could be enriched with other ingredients, like nuts or dried fruits and consumer perception could be evaluated.

Dry-fractionated proteins from the cake generated by the lipid extraction of durum wheat germ and bran could also be applied in meat analogs. In fact, in order to substitute meat, texturized vegetable proteins (TVP) were produced by extrusion-cooking, mimicking the fibrous structure and the consistency of meat, and used in plant-based alternatives. In this research, durum wheat cake proteins were used as a sustainable raw material in combination with pea protein isolates to produce TVP. The results emphasize the importance of evaluating both sensory and techno-functional properties of TVP, due to their important role in meat alternative formulated products. While oil absorption and protein digestibility were not influenced by dry-fractionated durum wheat cake protein addition, water absorption decreased. However, the sensory perception of moistness did not decrease, indicating that the change was small, therefore not perceived by the panel. Moreover, from a sensory point of view, no off-flavors or off-odors were found. This was a positive outcome given that by-products often represent a sensory challenge, that affects consumer acceptance. The findings support the innovative and sustainable use of durum wheat by-products in meat alternatives, demonstrating its successful incorporation as a novel application that was not previously explored. For a future perspective, the application of TVP in meat alternative products should be evaluated.

Finally, the application of dry-fractionated starch, usually considered as a co-product of protein production, was explored. Due to its gelling properties, dry-fractionated pea starch could be applied in confectionary products. The alternatives to substitute gelatin in jelly candies are being increasingly recurring, and in this scenario dry-fractionated pea starch could be advantageous. Rheological analysis revealed that jelly candies made with dry-fractionated pea starch exhibited a weaker microstructure and poor structural recovery after shear stress, indicating a fragile gel network. However, texture profile analysis showed that key parameters, such as hardness and gumminess, were similar to control samples made with corn starch and gelatin, suggesting that dry-fractionated pea starch can still deliver acceptable textural performance. Sensory sweetness was higher in the jelly candies produced with dry-fractionated pea starch, and that was positive since it could help to reduce the sugar in the formulation. This study demonstrates that using dry-fractionated pea starch in jelly candies could be a cost-effective and sustainable approach to valorizing it.

The common denominator of this doctoral thesis is the exploration of dry-fractionated properties in food products, demonstrating their potential applications in innovative food products, also by recovering by-products from the food industry. However, there are some drawbacks related to the flavor of those ingredients that in some cases can limit the purchases by consumers. Therefore, from a future perspective, technologies to reduce the legume flavor in dry-fractionated ingredients should be explored to obtain milder ingredients. One of the possible solutions could involve the fermentation and enzymatic treatment of dry-fractionated ingredients, aiming to reduce the off-flavors typically associated with the raw material. Second approach to this problem could be investigating the combination of isolates and dry-fractionated ingredients, to merge the techno-functional properties and to minimize the legume flavors in a simple way and test the boundaries of their applications. In this perspective, consumer tests could be carried out to understand the consumer perception towards these innovative foods.

The value of legumes in diet has been acknowledged since ancient times. Today, the use of technologies such as dry fractionation allows them to unlock their potential as ingredients into new forms, while preserving their properties and functionality. In this doctoral thesis, the creative incorporation of dry-fractionated ingredients into innovative food products proved that keys for a sustainable and healthy future may lie in our past.

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