Review

Received: 26 July 2023 Revised: 20 October 2023 Accepted article published: 27 November 2023 Published online in Wiley Online Library: 9 December 2023

(wileyonlinelibrary.com) DOI 10.1002/jsfa.13168

Techno-functional properties of dry-fractionated plant-based proteins and application in food product development: a review

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Abstract

Dry-fractionated protein concentrates are gaining attention because they are produced using a 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 on 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.[1,2] Indeed, the market size for protein ingredients is expected to grow at a yearly rate of 5.8% from 2023 to 2030.[3] Plant-based sources of protein include pulses, cereals, pseudocereals and nuts, whereas innovative protein sources are insects, fungi, microorganisms and microalgae.[2] 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),[1,2,4] as well as to produce alternatives to the animal-based food products (e.g.meat and dairy analogues).[1,5]

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%.[1,5,6] 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.[4,6] A comprehensive description of the methodologies and of the different technologies for wet extraction is provided elsewhere.[1,6,7]

A dry fractionation (DF) approach is largely more sustainable than wet extraction processes[6] because it consists of physical methods of concentration of the protein. In brief, DF operates

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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[4,8,9] 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.[9] Electrostatic separation is another DF technology, traditionally used in the mineral industry but recently implemented in the food sector.[8] This method relies on tribo-electric charging and, consequently, on the surface properties of flour particles.[10] 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.[9] Notably, electrostatic separation offers advantages when the aim is to fractionate dietary fibers.[11,12] A comprehensive description of DF technology is available in recent review articles.[4,8,9]

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.[4] Dumoulin *et al.*[13] 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.[14] 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%.[15] De Angelis *et al.*[16] 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.[13]

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 the 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).[17] 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

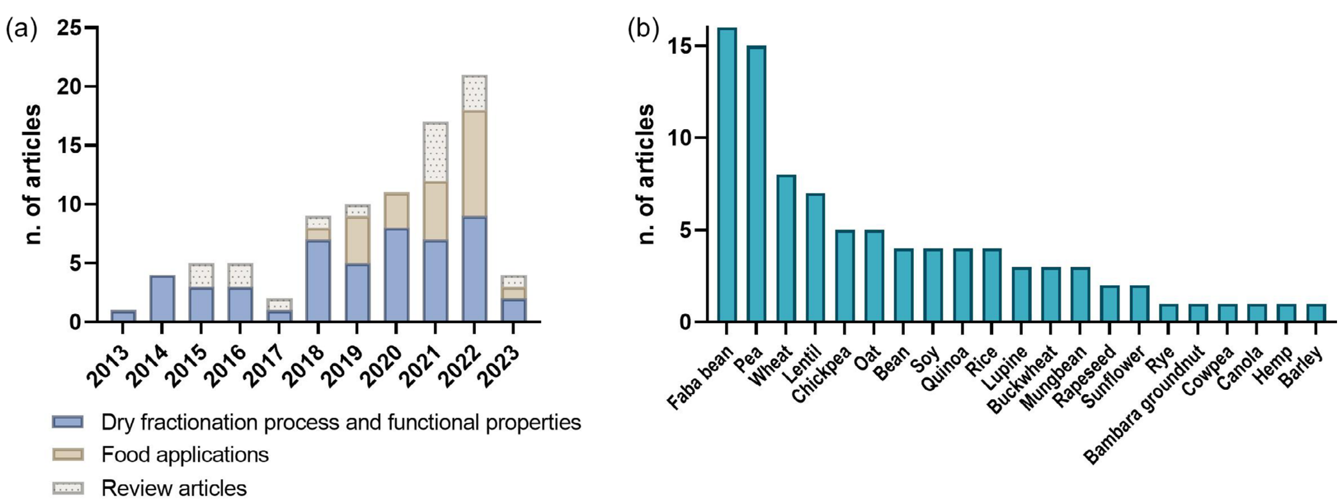

Figure 1. Number of published articles (a) and plant sources studied in the last 10 years (b) according to the literature search.

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. 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.

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.[9,18,19] 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[9] to obtain highly-added value ingredients, with the exception of raw material having an oil content higher than 35%.[6] 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 the problems related to the loss of the genetic resources and of the biodiversity heritage. Neglected and minor grains are well adapted to the local environments, require low input for their growth and tolerate stress in a way that they can be grown in marginal areas.[20] As described elsewhere, underutilized grains include, for example, varieties of cowpea, groundnuts, sesame, African locust bean and lima bean,[20] and the Apulian black chickpea.[21] 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 agro-ecological and socio-economic sustainability (https://agritechcenter.it). Moreover, this would also promote the protein diversification and increase the self-sufficiency of protein production.[22]

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.[23] Both surface and hydrodynamic properties, affected by the protein extraction technology, the botanical species considered and the protein concentration level,[1] 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.

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

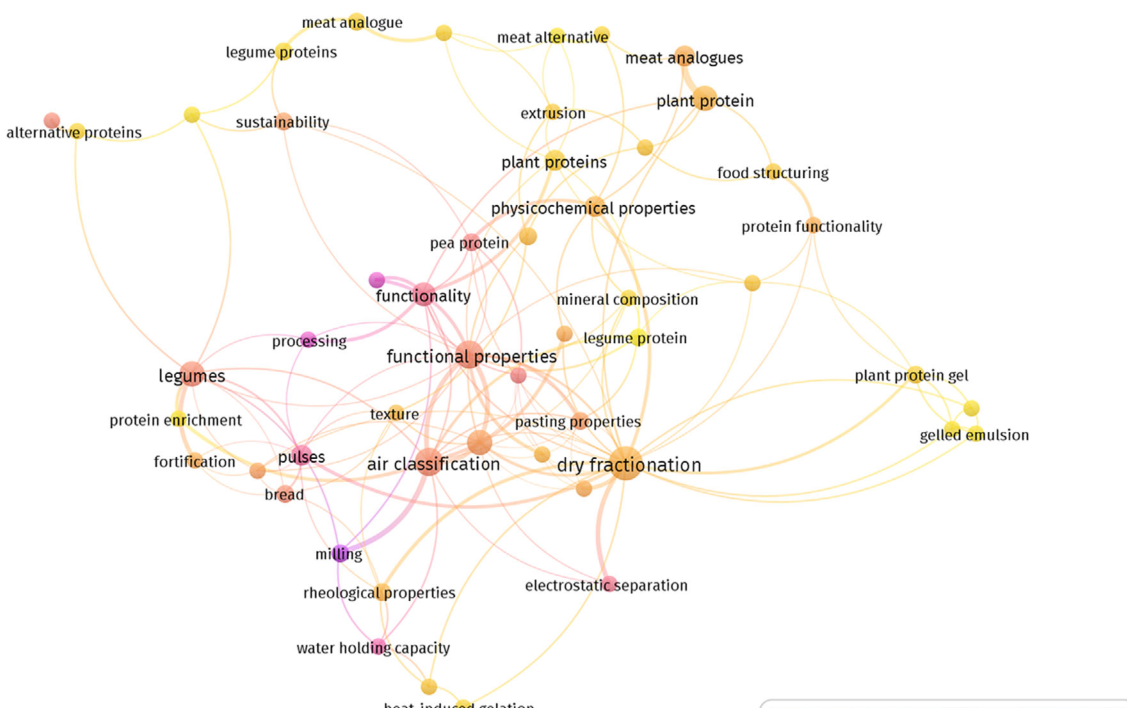

Figure 2. Network map of keyword co-occurrences colored according to year.

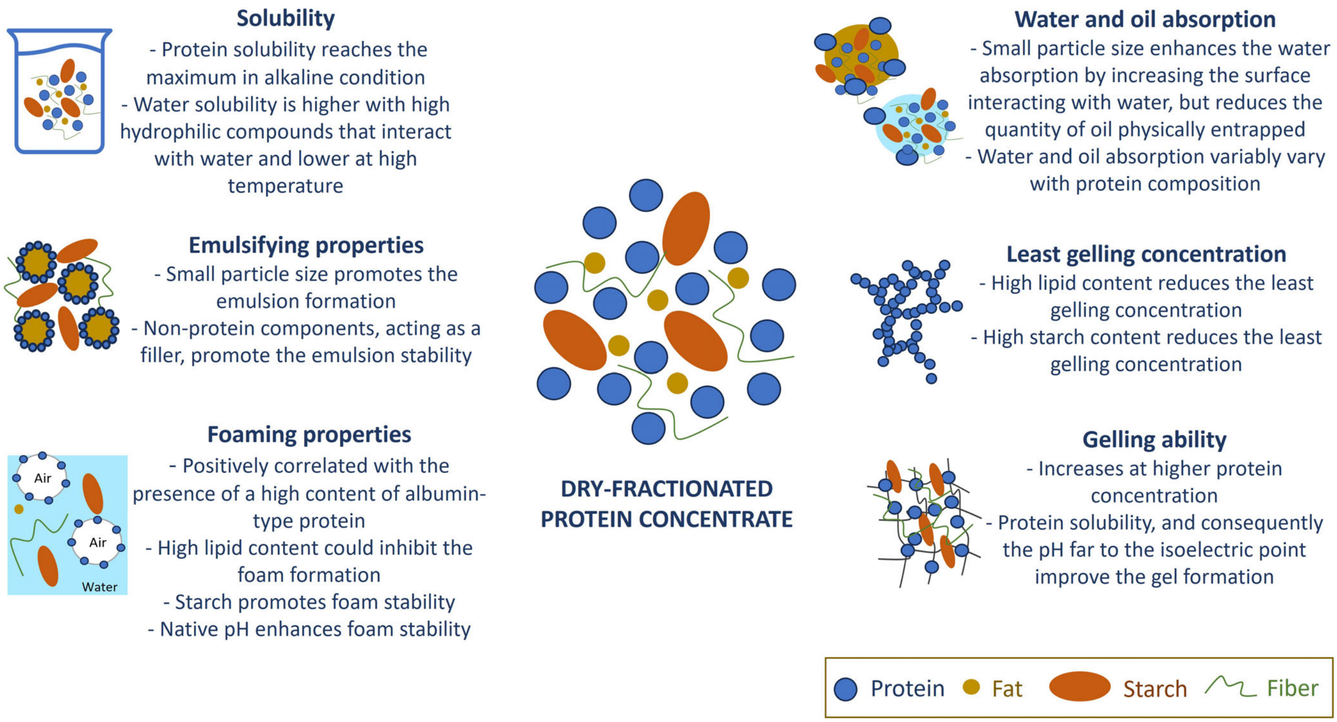

Figure 3. Highlights of the characteristics of the tecno-functional properties of dry-fractionated proteins.

different pH. Protein solubility reaches the maximum in alkaline conditions.[1,24,25] In particular, Saldanha do Carmo *et al.*[26] 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,[25] whereas dry-fractionated barley protein showed only 22.2% protein solubility at pH 8, which reached 91.9% at pH 11.[27] By contrast, superior solubility of dry-fractionated navy bean concentrate compared to the isolated one was reported in Tabtabaei *et al.*,[10] with the maximum value found at pH 8. These results confirm a great variability depending not only on the pH, but also on the protein composition and, consequently, on the botanical species.[26]

The protein extraction technology significantly influences the protein solubility. Vogelsang-O'Dwyer *et al.*[24] 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.[24]

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.[18,28] Therefore, WSI includes not only protein, but also all the hydrophilic compounds that interact with water.[26,28,29] In particular, soluble protein, starch and fibers contribute to the WSI, indicating an influence of the chemical composition.[18,28] 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,[18] quinoa,[28] and brown rice.[30] 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).[28] A high solubility might be exploited for all the food applications requiring the protein solubilization, such as non-acid protein beverages and milk substitutes (Fig. 4).

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.[1,26,31] 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 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.[32] 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.*[25] 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.*[28] 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.*[28] also suggested a significant influence of the particle size of the fine fraction (D_{50},

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	41	Bambara groundnut black-eye variety (*Vigna subterranea* L.)	29–33%	Minimum gelling concentration: 11–12% (w/w) Rheological properties
2018	27	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^{-1} OAC (at native pH, 5.1): 1.13 g g^{-1} LGC (at native pH, 5.1): 10%
2023	39	Commercial pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) protein concentrates	50–58%	EA: ~14.03–18.00 m^2 g^{-1} ES: ~ 5 min FC: ~85–97.8% FS after 60 min: 65–95% WAC: ~1.34–2.50 g water g^{-1} OAC: ~1.5–2.84 g oil g^{-1} LGC: 10–12%
2020	24	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	37	Two commercial Hemp (*Cannabis sativa* L.) protein concentrates	56–61%	PS and Pasting properties WAC: 0.8–1.3 g water g^{-1} LGC: 18–26%
2022	33	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	34	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	18	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^{-1} WSI: 21.38–27.30% WAC: 0.42–0.89 g water g^{-1} OAC: 0.45–0.53 g oil g^{-1}
2014	66	Lupine (*Lupinus angustifolius* L.)	54–59%	Foaming properties Viscosity
2022	38	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^{-1} LGC: ~8–10% Rheological properties
2022	35	Mung bean (*Vigna radiata* (L.) R. Wilczek)	23–24%	Rheological properties
2019	10	Pin-milled navy bean flour (*Phaseolus vulgaris* L.)	34–38%	EAI: 59.00% ES: 106.60% FC: 88.00% FS after 120 min: 41.5% WAC: 131.80% OAC: 82.4
2021	16	Green pea (*Pisum sativum* L.), yellow and red lentil (*Lens culinaris* Medik.)	54–62%	WAI: 3.91–4.04 g g^{-1} WSI: 24.56–26.98% WAC: 0.80 g water g^{-1} OAC: 0.49–0.51 g oil g^{-1}
2020	26	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^{-1} oil EAI: 12.32–18.80 m^2 g^{-1} ESI: 12.22–14.24 min FC: 26.56–54.84% WAC: 0.58–0.97 g water g^{-1}

Table 1. Continued

Year	Reference	Raw material	Concentration range	Techno-functional properties
				OAC: 1.11–1.14 g oil g^{-1}
				Gelling properties
2015	40	Yellow peas (*Pisum sativum* L.)	43%	Rheological properties
2013	67	Yellow peas (*Pisum sativum* L.)	51–55%	WAC: ~3.5 (w/w)
2020	28	Quinoa cv. Titicaca (*Chenopodium quinoa* Willd.)	13–24%	WAI: ~2–6.5 g g^{-1}
				WSI: ~8–17.5%
				EA: ~50–55%
				FC: 7 mL
				FS: 56%
				WHC: 2.31 g g^{-1}
				WAC: 0.97 g g^{-1}
				LGC: 12%
				Rheological and pasting properties
2018	68	Quinoa sweet varieties Atlas and Riobamba (*Chenopodium quinoa* Willd.)	32–33%	WAC and solubility at different temperatures
				Pasting properties
2016	11	Brown rice (*Oryza sativa* L.) bran	13–17%	WAC: 4.7 g g^{-1}
				OAC:6.7 g g^{-1}
2019	30	Germinated brown rice variety Chuchung	8%	WSI: 6.23%
				WAI: 2.23 g g^{-1}
				OAC: 92.93%
				Pasting properties
2019	69	Rice bran (*Oryza sativa* L. ssp. Indica)	20–27%	PS (pH 8): ~80%
2021	25	Commercial wheat and rye bran	31%	PS (pH 8): 43.6–75.50%
				WAC: 1.20 g water/g
				OAC: ~1 g oil g^{-1}
				Pasting properties

Abbreviations: PS, protein solubility; WAI, water absorption index; WSI, water solubility index; WAC, water absorption capacity; WHC, water holding capacity determined after 24 h; 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.

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.*[33,34] suggested that dry-fractionated lentil protein has a good potential in stabilizing an emulsion, corroborating the findings of Tabtabaei *et al.*,[10] 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.*[26] 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.*[24] and by Tabtabaei *et al.*,[10] 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.[10,35] Indeed, during the isoelectric precipitation, only the globulin fractions are recovered, whereas the majority of the albumin fraction is lost.[6,24,36] Albumins are superior for foaming because they form very strong interfacial films.[6] The foaming ability is also dependent on the botanical species; for example, using the same DF conditions, Saldanha do Carmo *et al.*[26] 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,[28] whereas the presence of starch has been reported to have a positive impact on foaming properties because of its ability to stabilize the foam.[27] Furthermore, other factors including the protein concentration[1] and pH [27] 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.[27]

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

For this reason, Ma *et al.*[1] 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.[36]

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.[1] WAC is also called water holding capacity (WHC) or water binding capacity, and as reviewed by Ma *et al.*[1] 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[28,30] and it also may be related to the presence of damaged starch in the protein fraction.[18] 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.[37] Moreover, other hydrophilic constituents besides proteins, such as fibers and polysaccharides, may have an influence on the water absorption.[18,26] Schlangen *et al.*[38] reported that the WAC of dry-fractionated mung bean, yellow pea and cowpea proteins was 2.15, 1.53 and 2.06 g g^{-1}, respectively, and could be linked to the different protein characteristics, rather than to the protein concentration. Instead, De Angelis *et al.*[18] reported WAC values in the range 0.42–0.89 g water g^{-1} in dry-fractionated lentils, chickpea and pea proteins, which increased to 2.98–3.48 g water g^{-1} 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[24] 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.[24] 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^{-1} in faba bean,[24] 1.13 g g^{-1} in barley fine fraction,[27] 1.0 g g^{-1} in wheat bran[25] and 0.82–0.91 g g^{-1} in navy bean,[10] whereas De Angelis *et al.*[18] reported a range of 0.45–0.53 g g^{-1} 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.[25] 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.[24,39] 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^{-1}) and high OAC (e.g. > 1 g g^{-1}) could be useful to enhance the cooking yield or prevent the cooking loss. However, it should be considered that a comparison data among studies is difficult because they depend on the methodology used for the determination.[1] This leads to concerns related to a 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[26,38,37]; 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.[38,40] The gelling properties of proteins can be assessed by determining the least (or minimum) gelling concentration (LGC).[1] However, rheological evaluations carried out by an oscillatory rheometer[24,28,38] or by a rapid visco amilograph[25,27,37] 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.*,[41] 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%.[37] de Paiva Gouvêa *et al.*[39] 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%.[28] Proteins have better gel ability compared to the other components (e.g. starch),[37] thus, LGC is negatively influenced by lipids and by starch. The latter competes for water during gelation.[28]

Interestingly, Vogelsang-O'Dwyer *et al.*[24] 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.*[24] 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.*,[37] who confirmed that the LGC was affected by the extraction technology and ingredient composition, reported a 18–26% LGC for dry-fractionated hemp and 12–26% for hemp extracted with aqueous extraction and isoelectric precipitation.

It was recently described how an 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.[42] 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.[37] Other factors affecting the gel formation include pH and consequently the protein solubility, as discussed by Silventoinen *et al.*,[27] 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).

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.[43,44] 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.[43] The utilization of dry-fractionated protein in bread and bakery products was previously investigated in different studies.[45-50] Moreover, an alternative application of the DF was studied by Ficco *et al.*,[51] 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.*,[12] 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.*[45] 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.*[46] prepared the mix of the previously studied protein[45] 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 protein were applied to partially substitute wheat flour with 20–30% w/w in wheat bread.[48] Interestingly, following the findings of their previous study,[47] 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.[47] 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.[48] 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

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	50	Gluten-free Focaccia flat bread	Pea (*Pisum sativum* L.)	Commercial protein concentrate	55%	5%
2022	49	Crackers	Faba bean (*Vicia faba* L.)	Commercial protein concentrate	~60%	40%
2021	48	Bread	Chickpea (*Cicer arietinum* L.)	Dry-fractionated chickpea and its sourdough	31%	20–30%
2020	46	Bread	Faba bean (*Vicia faba* L.)	Commercial protein concentrate combined with other protein ingredients	61%	5.72%
2020	47	Sourdough	Kabuli chickpea (*Cicer arietinum* L.)	Protein concentrate	~40%	12:6 g:g (protein: water)
2019	12	Bread	Wheat bran	Two batches of wheat bran	15–16%	2–10%
2019	45	Bread	Faba bean (*Vicia faba* L.)	Commercial protein concentrate and other protein ingredients	61%	15%
2018	51	Bread	Durum and soft wheat	Purple-pericarp and blue-aleurone	n. a.	30–40%
2022	54	Fresh high-protein hybrid pasta	Buckwheat and Faba bean (*Vicia faba* L.)	Dry-fractionated proteins	23–61%	4–13%
2021	52	Pasta	Faba bean (*Vicia faba* L.)	Dry-fractionated protein	59%	25%
2019	53	Protein-enriched gluten-free pasta	Oat and faba bean (*Vicia faba* L.)	Dry-fractionated protein	42–68%	~35%
2022	58	Meat analogues	Faba bean (*Vicia faba* L.)	Commercial protein isolates and dry- fractionated	55–80%	30–70%
2022	37	Meat analogues	Hemp (*Cannabis sativa* L.)	Commercial protein concentrate	61%	4:1 g:g (protein:maize starch)
2022	70	Meat analogues	Pea (*Pisum sativum* L.)	Commercial concentrate	49%	48%
2022	59	Meat analogues	Faba bean (*Vicia faba* L.)	Commercial protein	64%	40–70%
2021	57	Meat analogues	Faba bean (*Vicia faba* L.)	Commercial protein	64%	Water/Product feed rate ratio 3–5
2021	71	Meat analogues	Faba bean (*Vicia faba* L.)	Dry fractionated proteins	56%	38–42%
2021	60	Meat analogues	Pea (*Pisum sativum* L.)	Dry fractionated proteins	72%	4%
2020	55	Meat analogues	Pea (*Pisum sativum* L.)	Dry fractionated proteins in combination with oat protein	56%	35–70%
2022	63	Cheese analogues (spreadable)	Green pea (*Pisum sativum* L.)	Dry fractionated proteins combined with inulin-based emulsion-filled gel	56%	30%
2022	64	Dairy alternative (Fermented beverage)	Apulian black chickpea (*Cicer arietinum* L.)	Commercial dry-fractionated protein	56%	16.66%
2019	65	Dairy alternative (Yogurt-type)	Oat	Defatted dry-fractionated proteins	42%	15%
2022	72	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%

sensory quality. With this respect, after using a mixture design, De Angelis *et al.*[50] 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.*[49] 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.*[46] and Gangola *et al.*[52] evaluated the addition of dry-fractionated protein for the development of a nutritionally enhanced durum wheat-based pasta, whereas Duta *et al.*[53] worked on the formulation of gluten-free pasta.

Hoehnel *et al.*[54] 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.*[52] 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'.[53] 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 a 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[55,56] (i.e. protein with a fibrous structure mimicking the structure of the meat muscle). Texturized protein can be obtained through different structuring process, as comprehensively described elsewhere[56]; 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).[55,56] 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 avialable studies concerning the application of dry-fractionated proteins in meat analogues are all published no earlier than 2020. For example, De Angelis *et al.*[55] 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.[55] 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[37] and faba bean.[57-59] Zhu *et al.*[60] 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.*[57] 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.*[37] 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.*[58] who worked on faba bean obtained by DF and wet extraction. Nasrollahzadeh *et al.*[37] 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.*[57] 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.*[37] 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.[18,24,59] 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.*[59] 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 the soy production and consumption, other protein sources such as pulses have been recently investigated, as comprehensively reported in recent reviews.[61,62] 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.*[63] 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.[62]

Strategies to improve both texture and sensory quality of dairy alternatives include fermentation with selected starters, as investigated by Mefleh *et al.*,[64] 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.*[65] 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 the structure development during the 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 a 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 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.

AUTHOR CONTRIBUTIONS

DDA, VL, FC, AP and CS were responsible for conceptualization. DDA and VL were responsible for investigation. DDA was responsible for formal analysis. DDA and VL were responsible for writing the original draft. DDA, FC, AP and CS were responsible for reviewing and editing. FC and CS were responsible for resources. FC and CS were responsible for project administration.

ACKNOWLEDGMENTS

This research was carried out within (i) the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022); this manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them, and (ii) the Ministerial Decree no. 351 of 9th April 2022, based on the PNRR – funded by the European Union – NextGenerationEU – Mission 4 'Education and Research', Component 1 'Strengthening the offer of education services: from nurseries to universities' – Investment 4.1 'Extension of the number of research doctorates and innovative doctorates for public administration and cultural heritage' grant number H91I22000970007.

DATA AVAILABILITY STATEMENT

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

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