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

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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.

1. 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 the 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

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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 modulate 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 of this paper 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.

2. Materials and methods

2.1. 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. 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.

2.2. 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.

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

Table 1
List of the plant protein ingredients obtained with wet extraction and dry fractionation, and their proximate composition.

	Extraction Method	Proteins (g/100 g d.m.)	Lipids (g/100 g d.m.)	Ash (g/100 g d.m.)	Carbohydrates (g/100 g 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

protein ingredient.

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, IKA-Werke GmbH & Co. KG, Staufen, Germany) at 24,000 rpm for 3 min. Then, an amount of oil is added to the solution, and homogenized 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:

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

2.3. 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.

2.4. 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 point between G′ and G'' (Pa) was considered. The analysis was carried out in duplicate, preparing two distinct dispersions of each ingredient.

2.5. 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).

2.6. 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 and 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.

2.7. 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.

3. Results and discussion

3.1. Techno-functional properties of protein ingredients

Fig. 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 (Fig. 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 given in 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, the WE chickpea (Supplier 1) had a WSI of 32.18% while the 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 (Fig. 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 the 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, 2024d) requires the knowledge of the water absorption. Moreover, a high WAC is reported to be desired in meat analogs 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.

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 (Fig. 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 de Paiva Gouvêa et al. (2023) and

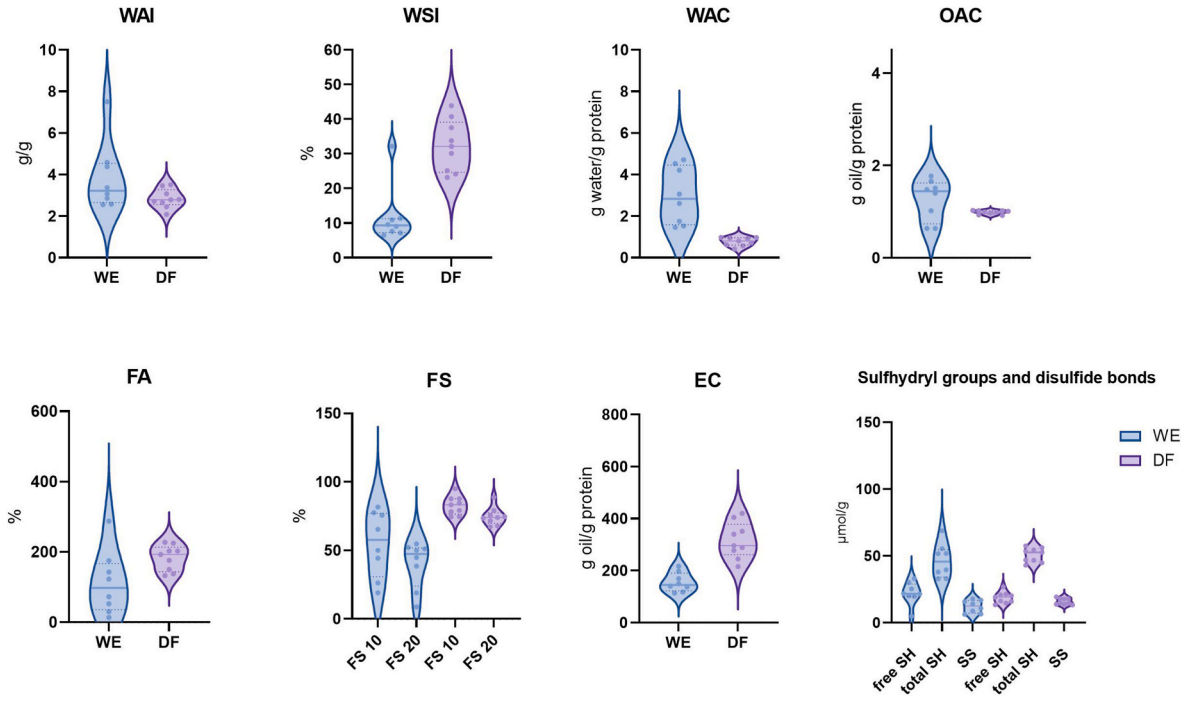

Fig. 1. Violin plot illustrating the distribution of the techno-functional properties of wet-extracted (WE) and dry-fractionated (DF) ingredients. WAI: water absorption capacity; WSI: water solubility index; OAC: oil absorption capacity; FA: foaming ability; FS: foaming stability; EC: emulsion capacity, 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.

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

		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
	WE	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.

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 Fig. 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

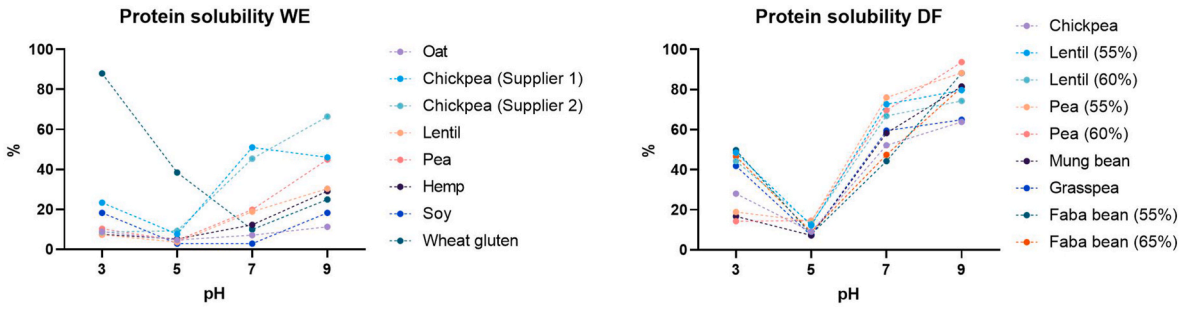

Fig. 2. Protein solubility (expressed as percentage) of wet-extracted (WE) and dry-fractionated (DF) ingredients determined at different pH conditions.

ingredients might be preferable, whereas for fermented or acidic beverages, we could hypothesize a similar behavior between DF and WE ingredients.

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 min. 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, 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). Fig. 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, 13.75, 52.50% respectively). Shevkani et al. (2014) explained that lipids are more surface active 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).

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).

3.2. Rheological evaluation of the gelling behavior

The gelation behavior of the ingredient dispersions subjected to a temperature ramp is depicted in Fig. 3. DF ingredients showed uniform behavior during the temperature sweep analysis, whereas a 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 (Fig. 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

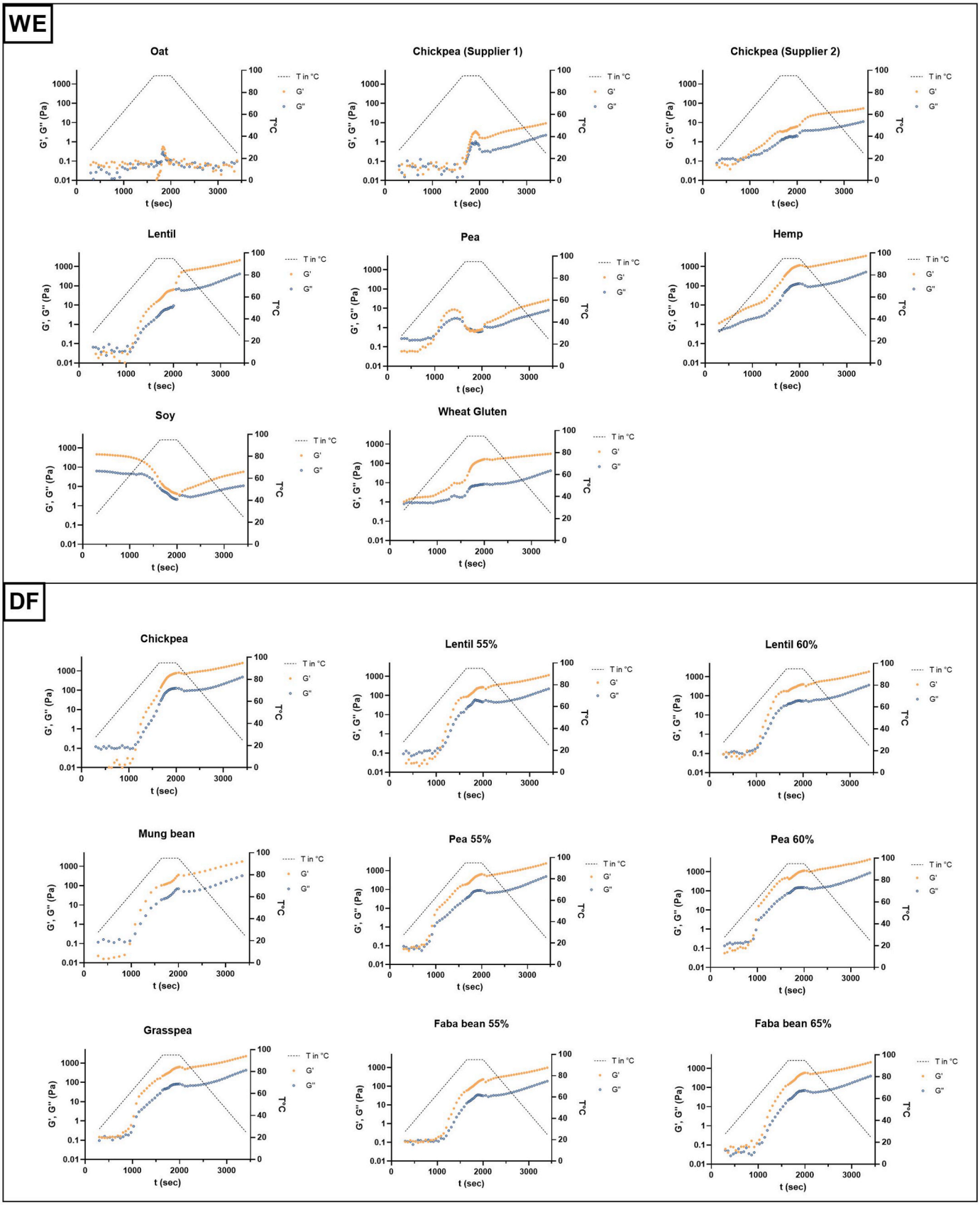

Fig. 3. Storage modulus (G′, Pa) and loss modulus (G″, Pa) of wet-extracted (WE) and dry-fractionated (DF) ingredients dispersions as a function of the temperature (T, °C).

Table 3
Mean of final values of G′ and G″ of dry-fractionated (DF) and wet-extracted (WE) plant protein ingredients.

	Sample	G' (Pa)	G″ (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.

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.

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

The total, free SH and SS bonds of the protein samples are presented in 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 (Fig. 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) plant protein ingredients.

		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 $p < 0.05$.

3.4. Exploratory analysis on the secondary structure of proteins

The FTIR spectra acquired from WE and DF samples are shown in Fig. 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., (2012) 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

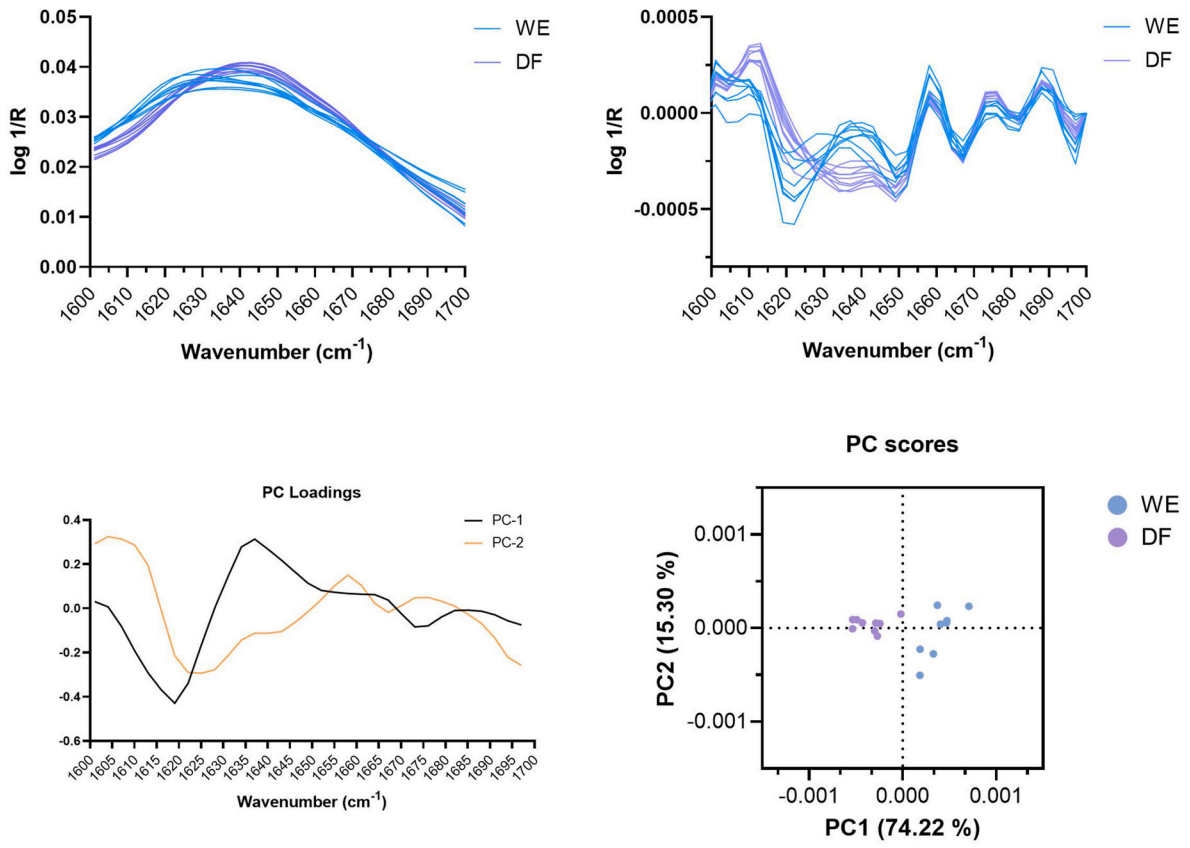

Fig. 4. Results of the exploratory analysis of the secondary structure. Top left: raw FTIR spectra within the amide 1 region; top right: processed spectra using the Savitzky-Golay second order derivation and two smoothing points; bottom left: loading plot of the Principal Component Analysis carried out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

results of the PCA (Fig. 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., 2012; Tang et al., 2021; Nasrollahzadeh et al., 2023). By contrast, a positive contribution to PC1 is given by the regions between 1631 and 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., 2012; 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.

4. Conclusion

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.

Author contributions

Davide De Angelis: Conceptualization, Investigation, Resources, 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.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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