

Original Article

Dry fractionation as a promising technology to reuse the physically defected legume-based gluten-free pastaDavide De Angelis,^{1*} Antonella Pasqualone,¹ Luigi Manfredi,² Ignazio Allegretta,¹ Roberto Terzano¹
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Summary Dry fractionation was applied to the legume-based pasta (yellow lentils:whole rice 90:10 w:w) discarded for physical defects. After the air classification, the fine fraction showed a 33% increment of the protein content compared to the raw material, with a 21% yield. The scanning electron micrographs revealed the presence of protein–starch complexes and broken starch granules which led to a low protein separation efficiency. The fine fraction showed interesting nutritional features due to the high concentration of the essential micronutrients Zn (43.3 mg kg⁻¹) and Fe (72.6 mg kg⁻¹). However, also the alpha-galactosides were enriched into the same fraction. The two fractions, fine and coarse, were both characterised by elevated water absorption capacity, with significantly higher values in the fine one. Finally, the gelling capacity varied among the fractions, being influenced by the protein content. Overall, these ingredients could be used to fortify the protein and the essential mineral contents of bakery products, sauces, and creams.

Keywords air classification, dry-fractionated protein, functional properties, legume protein, lentil pasta, mineral composition, plant-based ingredient.

Introduction

Pasta is one of the most iconic foods produced and consumed in Italy and it is made by mixing durum wheat semolina and water. In recent years, new categories of pasta have been developed using novel ingredients, especially to satisfy the needs of coeliac patients. One of the most promising products is legume-based pasta, whose market is expected to more than double its size in 2025, reaching 2400 million dollars (Research & Market, 2020). However, as for all the food productions, also the pasta industry generates by-products and waste. The latter includes defected dry pasta arising from (i) occasionally misled drying processes, causing deformations, cracking, or non-enzymatic browning and (ii) accidental ruptures during or after the packaging operations (Hui, 2006). The quantity of defected legume-based pasta produced during the manufacturing process can be quantified in about 15% of the production, as per direct information given by the main producers. This value varies according to the type of pasta produced, with higher

percentages for long-cut and sheeted than for short-cut pasta. Defected pasta can be re-milled and reused in the same production process, but only at low addition levels since higher amounts affect the product quality. Therefore, although defected pasta is a valuable material discarded only for physical non-conformities, it is commonly destined for animal feeding, such as meat quail (Santos *et al.*, 2020) or broilers (Santos *et al.*, 2018) without any particular interest in its revalorisation. Indeed, while the incorporation of by-products from other food supply chains in pasta manufacturing is quite well investigated (Pasqualone *et al.*, 2015, 2017), the reuse of defected fresh pasta for innovative food applications has been rarely proposed. The isolation of food ingredients, such as gluten (Ghorbel *et al.*, 2010) and starch (Ellouzi *et al.*, 2015), by wet extraction (which is a very water-consuming process), has been investigated. In the non-food sector, instead, the production of biodegradable packaging (Ellouzi *et al.*, 2019) has been proposed.

Modern research strategies involve the utilisation and exploitation of the resources in an efficient way, minimising food loss and enhancing the sustainability of food production, as strongly encouraged by policy programs (EC Food 2030 Expert Group, 2018). These

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efforts should be even more intense due to the possible consequences of the Covid-19 pandemic on food security, which might leave millions of people hungry in the next years (FAO, 2020). The scarce utilisation of discarded pasta to obtain valuable food ingredients is related to the scarce sustainability of the extraction procedures proposed so far. Moreover, although the reuse of defective pasta for the production of the same product in the same productive site is ruled in Italy (DPR 187/2001, 2001), there is no specific legislation concerning the reutilisation of this waste for other food products. In the latter case, the good manufacturing practices (GMP) and the HACCP plan during the production process, as well as the current regulations on by-products and wastes (European Community, 2008, Directive 2008/98/EC) would apply.

In this scenario, the dry fractionation process can be the best tool for reusing defected pasta by recovering protein and starch ingredients in a sustainable way. Dry fractionation technology is gaining interest over the years because of its simplicity, without involving any use of water and chemicals (Schutyser *et al.*, 2015). Dry fractionation is commonly applied to pulses (De Angelis *et al.*, 2021b), and it is based on the separation, by solely physical means, of a coarser fraction, mainly composed of starch granules, and a finer fraction, mainly composed of protein particles. Recent research reported the suitability of this process for the valorisation of olive pomace (Lammi *et al.*, 2018), as well as rapeseed and sunflower meals (Laguna *et al.*, 2018). To the best of the authors' knowledge, the application of dry fractionation to pasta industry by-products has not been proposed yet.

Therefore, the aim of this study was to investigate the use of dry fractionation technology for the upcycling of discarded dry pasta, affected by defects of physical nature. The influence of the process on the nutritional composition, the physicochemical and functional properties of the fractions has been evaluated, to assess their potential use as food ingredients.

Materials and methods

Physically defected pasta and dry fractionation process

The physically defected legume-based gluten-free pasta (DLP) were kindly provided by Andriani S.p.A (Gravina in Puglia, Italy) and it was made with 90% of yellow lentils and 10% of whole rice. The DLP was milled until a granulometry <0.2 mm, as reported in Pasqualone *et al.* (2021) and subjected to dry fractionation in collaboration with Innovaprot srl (Gravina in Puglia, Italy). The whole process was carefully described in De Angelis *et al.* (2021a) and it consists of the separation of the micronised flour (M_F) into two fractions: a coarse fraction (C_F) and a fine one (F_F). The yield was calculated by dividing the quantity

of each fraction collected by the total weight of the micronised flour and expressed as a percentage. The protein separation efficiency (PSE) was calculated as:

$$PSE = \frac{P_{xf} Y_{xf}}{P_{mf}}$$

where P_{xf} is the protein content of the fraction considered (% on the dry matter), Y_{xf} is the yield of the fraction considered (%), and P_{mf} is the protein content of the micronised flours (% on the dry matter).

Scanning electron microscopy

The morphology of the grains of each fraction was studied using a field emission gun scanning electron microscope (FEG-SEM) Zeiss Sigma 300 VP (Zeiss Oberkochen, Germany). Micrographs were acquired with a secondary electron detector (SE) using an accelerating voltage of 10 kV and a working distance of 3–5 mm. For the analysis, an aluminium stub was covered with a carbon disk, on which the powdered sample was deposited, and the specimens were carbon coated by sputtering.

Proximate composition

The official methods AOAC 979.09, 923.03, and 925.10, were used to determine the protein content (total nitrogen $\times 6.25$), ash, and moisture content, respectively (AOAC International, 2006). The lipid content was determined after a Soxhlet extraction using diethyl ether (Merck KGaA, Darmstadt, Germany) as solvent (AOAC International, 2006). Total, soluble, and insoluble dietary fibre content was determined by the enzymatic–gravimetric procedure according to the AOAC Official Method 991.43 (AOAC International, 2006). Total carbohydrates content was calculated as difference [100–(protein + lipids + ash)]. Damaged starch content was determined according to the AACC method 76.31 (AACC, 1992). The analyses were carried out in triplicate.

Soluble sugars content

Soluble sugars verbascose, stachyose, raffinose, and sucrose were determined using high-performance liquid chromatography (1260 Infinity, Agilent Technologies, Santa Clara, USA), equipped with a Refractive Index Detector (1260 Infinity RID), according to the method previously described in De Angelis *et al.* (2021b). The results were expressed as mg g^{-1} on a dry matter basis. The analysis was carried out in triplicate.

Determination of mineral composition

The mineral composition (P, S, Cl, K, Ca, Mn, Fe, Ni, Cu, and Zn) of the legume-based gluten-free pasta by-products was studied using total-reflection X-ray

fluorescence (TXRF) spectroscopy, according to De Angelis *et al.* (2021b). Briefly, 100 mg of sample were suspended with 5 mL of a 1% Triton X-100 solution (Merck KGaA, Darmstadt, Germany). A volume of 10 μL of a 1000 g L^{-1} Ga standard solution (Trace-CERT[®], Merck KGaA, Darmstadt, Germany) was added as internal standard and the suspension was vortexed for 5 min. The suspension was finally treated in an ultrasonic bath for 15 min. Then, 10 μL of suspension were transferred onto a quartz sample carrier and left to dry at 50°C on a heating plate. The analyses were carried out in triplicate using an S2Picofox TXRF spectrometer equipped with a Mo source (50 kV, 600 μA) and an XFlash[®] Silicon Drift Detector of 30 mm^2 (Bruker Nano GmbH, Germany) setting a live time of 1000 s.

Physicochemical and functional properties

Bulk density (BD), water absorption index (WAI), water solubility index (WSI), water absorption capacity (WAC), and oil absorption capacity (OAC), of the fractions were determined according to the procedures described by Summo *et al.* (2019). The foaming activity (FA) and foaming stability at 10 (FS10) and 20 (FS20) min were determined according to Xiong *et al.* (2018) with some modifications. One gram of fraction was suspended in 20 mL of distilled water at 20°C and then homogenised in a beaker with an ultra-turrax (model T-25, IKA Werke GmbH & Co. KG, Staufen, Germany) at 13 500 rpm for 90 s. The suspension was transferred into a graduated cylinder and the beaker was washed with 5 mL of water, then added into the cylinder. FA is determined as the percent ratio between the foam volume at 1 min and the initial volume of samples, whereas the FS10 and FS20 as the percent ratio between the foam volume at 10 and 20 min and the initial foam volume. The analyses were carried out in triplicate.

Gelling behaviour

The least gelling concentration (LGC) of the micronised DLP and of their air-classified fractions was determined according to Boye *et al.* (2010) with few modifications. Rising concentrations of fractions (from 2% to 20% w/v) were dispersed with 5 mL of distilled water in centrifuge tubes. The samples were vortexed for 2 min and heated in a water bath at 100°C for 1 h. The tubes were cooled down with cold water and then stored overnight at 4°C. The tubes were inverted and gently tapped to determine whether the gel formation occurred or not. Following the indications of Boye *et al.* (2010), the LGC was identified as the concentration below which no self-supporting gel was formed. The analysis was carried out in duplicate.

Statistical analysis

Data were subjected to one-way analysis of variance ANOVA followed by Tukey's honestly significant differences test for multiple comparisons at a significance level $\alpha = 0.05$ using the Minitab 17 Statistical Software (Minitab Inc., State College, PA, USA).

Results and discussion

Dry fractionation process, morphology, and proximate composition

The proximate composition, together with the yield and the PSE, are reported in Table 1. The DLP M_F reflects a typical composition of the initial raw material (Boye *et al.*, 2010; De Angelis *et al.*, 2021b). The dry fractionation process allowed to obtain a F_F enriched in protein (33% protein increment, corresponding to 34.61 $\text{g } 100 \text{ g}^{-1}$ of protein), with a yield of 21%. In our previous studies concerning the dry fractionation of pulse flours, using the same set-up of the air classification, the yield of the F_F was comparable, but the protein content was considerably higher when compared to the DLP F_F , reaching about 50 $\text{g } 100 \text{ g}^{-1}$ (De Angelis *et al.*, 2021a,b). To better understand the reasons behind the lower protein enrichment, the morphology of the raw materials and of the fractions was studied by scanning electron microscopy. The micrographs are shown in Fig. 1. The raw material appeared as a mix of whole starch granules, small fragments of proteins and broken starch, and gelatinized starch granules which led to the formation of fragments of a complex protein matrix of various dimensions. This is evident from the micrographs showing the C_F , in which are recognisable starch granules embedded into the protein–starch complex (red arrows) alongside isolated granules (yellow arrows). Finally, in the F_F , the presence of broken starch granules as well as small fragments of complexes is relevant. The presence of such complexes, imputable to the extrusion-cooking process usually adopted to prepare DLP (Dogan *et al.*, 2013; Kumar *et al.*, 2017), makes the dry fractionation more difficult compared to a native legume flour (De Angelis *et al.*, 2021b). The extrusion cooking process, indeed, causes physicochemical and structural modifications of the flour constituents, such as starch gelatinisation (Pasqualone *et al.*, 2021). The latter leads to the loss of the natural starch conformation, hindering the separation of the starch granules from the protein bodies and lowering the PSE (De Angelis *et al.*, 2021b).

However, despite the low protein content, the defective pasta is a low-cost raw material, therefore, even a moderate protein increment achieved by dry fractionation could be valuable under the nutritional,

Table 1 Proximate composition (mean \pm standard deviation) expressed as g 100 g⁻¹ on dry matter of the defected pasta before (M_F) and after separation by dry fractionation in coarse (C_F) and fine (F_F) fractions, together with the Yield % and the protein separation efficiency (PSE) of the two fractions

	M _F	C _F	F _F
Protein	26.09 \pm 0.12 ^b	23.02 \pm 0.06 ^c	34.61 \pm 1.42 ^a
Lipids	1.02 \pm 0.00 ^b	0.79 \pm 0.02 ^b	1.93 \pm 0.32 ^a
Total carbohydrates	70.26 \pm 0.03 ^b	73.89 \pm 0.02 ^a	60.48 \pm 1.78 ^c
Total dietary fibre	6.17 \pm 0.14 ^b	5.61 \pm 0.11 ^c	6.63 \pm 0.18 ^a
Soluble dietary fibre	1.64 \pm 0.10 ^a	1.38 \pm 0.09 ^b	1.72 \pm 0.13 ^a
Insoluble dietary fibre	4.53 \pm 0.15 ^b	4.23 \pm 0.15 ^c	4.91 \pm 0.11 ^a
Damaged starch	8.22 \pm 0.30 ^{ab}	7.31 \pm 0.42 ^b	8.52 \pm 0.45 ^a
Ash	2.63 \pm 0.15 ^b	2.30 \pm 0.06 ^b	2.99 \pm 0.20 ^a
Yield%		79.0	21.0
PSE%		69.4	27.9

Different superscript letters in the same row indicate significant differences according to Tukey's test at $\alpha = 0.05$.

technological, and economical points of view. The dietary fibre was mainly composed of the insoluble fraction as previously reported for legumes and, in particular, for lentil flour (Pasqualone *et al.*, 2021). The dry fractionation led to an enrichment of the dietary fibre in the fine fraction. Protein bodies are surrounded by a fibre-rich cell wall (Pelgrom *et al.*, 2015; Schutyser *et al.*, 2015), which is finely milled during the micronisation process and migrate into the fine fraction. The finer fraction was also characterized by a higher ash content than the raw material, meaning a higher concentration of mineral compounds. This trend can be explained by the physical segregation of the mineral elements located in the different areas of legume seed (Kruger *et al.*, 2015) which then easily shift into the fine fraction. By contrast, the C_F, which accounted for the highest yield during the separation (79%), showed a significantly lower protein content compared to the raw material and, consequently, a higher carbohydrates content. Again, although the differences were significant, they were smoother than what was observed in native pulse flours (De Angelis *et al.*, 2021b).

Mineral composition

The mineral elements' composition is reported in Table 2. The macroelements quantified were P, S, Cl, K, and Ca, whereas the micronutrients were Mn, Fe, Ni, Cu, and Zn. Traces of selenium were detected; however, Se concentration was below the limit of quantification. The composition of the raw material

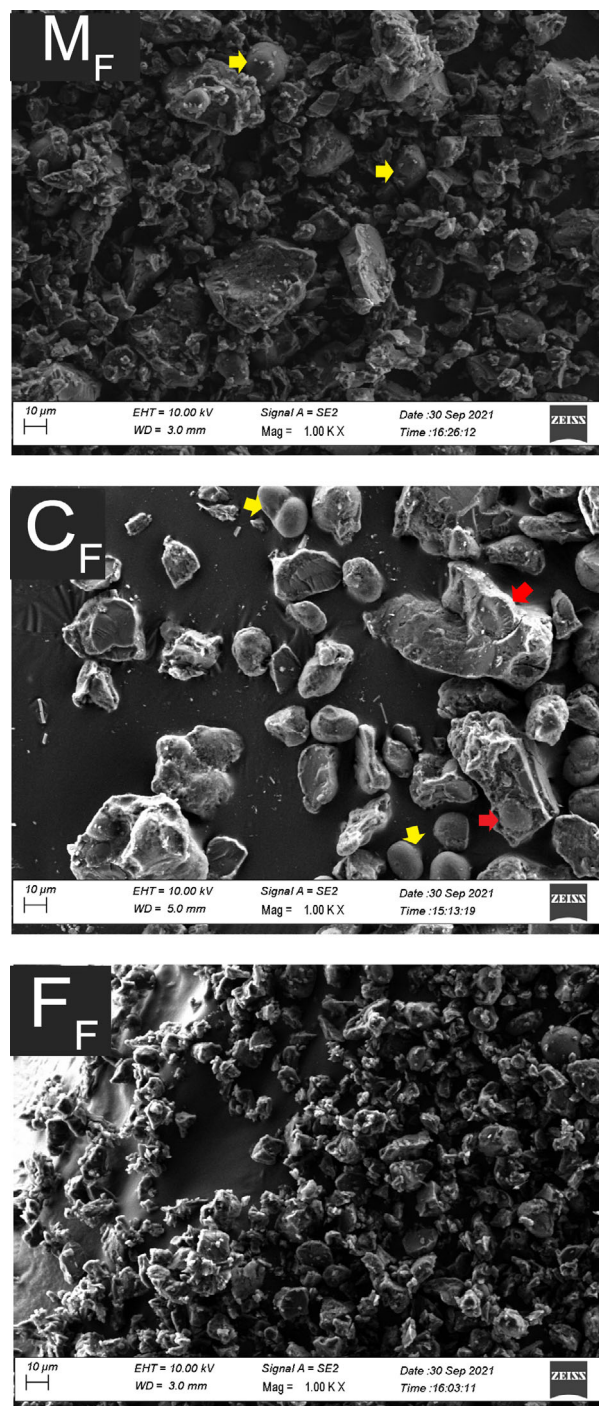


Figure 1 Scanning electron micrographs of the defected pasta before (M_F) and after separation by dry fractionation in coarse (C_F) and fine (F_F) fractions. Red arrows indicate protein-starch complex; yellow arrows indicate isolated granules.

follows the one observed for the native lentil flour (Wang & Daun, 2006; De Angelis *et al.*, 2021b). In particular, the most abundant macronutrient was K, followed by P and S. The high K content can help to lower the blood pressure in hypertensive and normotensive population, reducing stroke and other cardiovascular diseases (Lanham-New *et al.*, 2012). Whereas patients with impaired kidney functions can suffer the high K content of such ingredients. Overall, K intake in Western societies is normally in the range of 1.6–5.9 g/d (Lanham-New *et al.*, 2012). Iron, Zn, and Mn were the principal micronutrients.

The effect of the dry fractionation process on the elemental composition was significant, leading to important nutritional effects. Both macro- and micronutrients significantly diminished in the C_F, while a significant increase of the concentration of mineral elements in the fine protein fraction was observed. The shifting of the mineral elements after the air classification was previously reported (De Angelis *et al.*, 2021b). Interestingly, the shifting was different considering the macro- and the micronutrients. Indeed, a 9.4% increase of the total macronutrients was recorded, whereas the content of the total micronutrients increased by 48.6%. This was principally due to the enrichment of the essential elements Fe and Zn by 75.6% and 47.1%, respectively, with respect to the starting raw material. This result emphasizes the importance of dry fractionation in producing a protein concentrate that can be used as a valuable ingredient for different nutritional aspects. Indeed, considering 30 g of dry F_F protein a reasonable amount of ingredient to be included in a portion of a dairy (Mattice & Marangoni, 2020) and a meat alternative (Kyriakopoulou *et al.*, 2019) the contribution to the recommended nutrient intakes (WHO, 2004) would reach

Table 2 Elemental composition (mean ± standard deviation) expressed as mg kg⁻¹ on dry matter of the defected pasta before (M_F) and after separation by dry fractionation in coarse (C_F) and fine (F_F) fractions

	M _F	C _F	F _F
P	3649 ± 34 ^b	3329 ± 50 ^c	4484 ± 108 ^a
S	1690 ± 67 ^b	1645 ± 22 ^b	2162 ± 54 ^a
Cl	633 ± 16 ^a	630 ± 20 ^a	620 ± 16 ^a
K	9819 ± 105 ^a	9290 ± 181 ^b	9925 ± 150 ^a
Ca	270 ± 3 ^b	232 ± 6 ^c	372 ± 5 ^a
Mn	13.8 ± 0.2 ^b	12.4 ± 0.5 ^c	20.1 ± 0.3 ^a
Fe	41.4 ± 3.9 ^b	36.0 ± 1.6 ^b	72.6 ± 2.2 ^a
Ni	2.8 ± 0.2 ^{ab}	2.7 ± 0.1 ^b	3.0 ± 0.1 ^a
Cu	8.5 ± 0.2 ^b	7.1 ± 0.02 ^c	11.4 ± 0.2 ^a
Zn	29.4 ± 0.03 ^b	25.9 ± 0.4 ^c	43.3 ± 0.7 ^a

Different superscript letters in the same row indicate significant differences according to Tukey's test at $\alpha = 0.05$.

1.6% of Ca for adults, 26.5% and 18.5% of Zn for adult females and males, respectively, as well as 7.4% and 15.9% of Fe for adult females and males, respectively (Table 3). Eventually, the bioavailability of the minerals should be assessed to evaluate the real absorption of such compounds during digestion.

Soluble sugars

The soluble sugars content is reported in Table 4. Stachyose was the main oligosaccharide identified in the raw material, followed by raffinose, and verbascose. The dry-fractionation had a significant influence on the content of the alpha-galactosides, leading to a shifting of such components into the F_F. Compared to the raw material, the F_F showed a significantly higher verbascose and stachyose content, whereas the raffinose was significantly higher only in comparison with the C_F. By contrast, sucrose content appeared to be not affected by the air classification. Stachyose, being the most abundant oligosaccharide in lentils (Liu *et al.*, 2020; De Angelis *et al.*, 2021b), is therefore normally present in the DLP used in this study.

Alpha-galactosides are grouped into the raffinose family oligosaccharides, a class of indigestible carbohydrates that are responsible for the flatulence commonly associated with legumes consumption (Tahir *et al.*, 2012; Liu *et al.*, 2020). However, oligosaccharides aid the growth of the intestinal microbiota (Singh *et al.*, 2017) and they contribute to reducing the glycaemic index of the pasta, asserting a positive role in diabetic/anti-obese diet (Goñi & Valentin-Gamazo, 2003). The increase of the alpha-galactosides, as well as of other antinutritional factors, in the dry-fractionated protein fraction was previously

Table 3 Percentage of the WHO/FAO recommended nutrient intake levels (RNI) for Ca (prescribed for the European Union), iron (assuming 10% absorption), and zinc (assuming moderate bioavailability)

	RNI (mg/day)	% of RNI with 30 ^d g of M _F	% of RNI with 30 ^d g of F _F
Ca			
Females/males ^a	700	1.2	1.6
Fe			
Females ^b	29.4	4.2	7.4
Males ^b	13.7	9.1	15.9
Zn			
Females ^c	4.9	18.0	26.5
Males ^c	7	12.6	18.5

^aMature adults.

^b18+ years old.

^c18–65 years old.

^dReasonable quantity of the dry ingredient to be used in dairy and meat alternatives.

Table 4 Sugars contents (mean \pm standard deviation) expressed as mg g⁻¹ on dry matter of the defected pasta before (M_F) and after separation by dry fractionation in coarse (C_F) and fine (F_F) fractions

	M _F	C _F	F _F
Verbasose	12.62 \pm 0.21 ^{ab}	12.05 \pm 0.55 ^b	13.55 \pm 0.87 ^a
Stachyose	29.07 \pm 0.32 ^b	27.58 \pm 1.02 ^c	34.96 \pm 0.37 ^a
Raffinose	19.67 \pm 0.65 ^{ab}	18.90 \pm 0.52 ^b	20.77 \pm 0.57 ^a
Sucrose	31.70 \pm 1.40 ^a	30.51 \pm 0.90 ^a	29.50 \pm 1.57 ^a

Different superscript letters in the same row indicate significant differences according to Tukey's test at $\alpha = 0.05$.

reported (De Angelis *et al.*, 2021b). These findings suggest that the utilisation of such fractions for food production should be preferably coupled with technologies able to reduce their contents, such as extrusion-cooking (Pasqualone *et al.*, 2020) or fermentation (Xing *et al.*, 2020).

Moreover, it should be noted that the alpha-galactosides content of DLP is lower than the native lentil flour (De Angelis *et al.*, 2021b), due to the effect of pasta manufacturing phases, namely the extrusion-cooking and drying stage. At the same time, the concentration of the soluble sugars depends on the variety and on the growing environment (Tahir *et al.*, 2012).

Physicochemical and functional properties

The physicochemical (BD, WAI, and WSI) and functional (WAC and OAC) properties of the DLP and of its relative fractions are reported in Table 5. The dry fractionation significantly influenced the physicochemical properties, leading to a decrement of the BD in the F_F and to an increase of the density in the C_F. BD is the mass per occupied volume (Summo *et al.*, 2019) and the higher values of the C_F are synonyms of heavier particles with larger dimensions, as shown in Fig. 1, which are classified during the dry fractionation process (Pelgrom *et al.*, 2015; De Angelis *et al.*, 2021b).

The WAI and WSI are two physicochemical properties related to the physical state of starch granules and their swelling capacity (Du *et al.*, 2014; Summo *et al.*, 2019; De Angelis *et al.*, 2021b). The WAI and WSI significantly varied among the fractions, indicating an influence of the dry fractionation process. In particular, the F_F showed a lower WAI compared to both the raw material and the C_F and a higher WSI. Indeed, WAI and WSI are inversely correlated as highlighted in previous studies (Summo *et al.*, 2019; De Angelis *et al.*, 2021b). Being connected to the physical state of the starch, it is reasonable to assume that the higher value of WSI found in the F_F should be associated with the highest presence of damaged starch found in this fraction (8.52%), as well as to the increase of the content of protein which is soluble in water.

Table 5 Physicochemical and functional properties (mean \pm standard deviation) of the defected pasta before (M_F) and after separation by dry fractionation in coarse (C_F) and fine (F_F) fractions

	M _F	C _F	F _F
Bulk density (g mL ⁻¹)	0.80 \pm 0.02 ^b	0.91 \pm 0.00 ^a	0.69 \pm 0.02 ^c
Water absorption index (g/g)	4.23 \pm 0.15 ^a	4.46 \pm 0.01 ^a	3.74 \pm 0.12 ^b
Water solubility index (%)	16.76 \pm 0.15 ^b	14.22 \pm 0.02 ^c	25.33 \pm 0.50 ^a
Water absorption capacity (g water g ⁻¹ flour)	1.16 \pm 0.00 ^b	1.15 \pm 0.02 ^b	1.22 \pm 0.02 ^a
Oil absorption capacity (g oil g ⁻¹ flour)	0.39 \pm 0.02 ^a	0.26 \pm 0.01 ^b	0.41 \pm 0.02 ^a
Foam activity (%)	38.33 \pm 2.74 ^b	42.14 \pm 3.93 ^b	54.00 \pm 4.18 ^a
Foam stability at 10 min (%)	93.36 \pm 5.45 ^{ab}	95.83 \pm 5.61 ^a	85.21 \pm 4.94 ^b
Foam stability at 20 min (%)	90.48 \pm 4.82 ^a	91.87 \pm 5.58 ^a	85.21 \pm 4.94 ^a

Different superscript letters in the same row indicate significant differences according to Tukey's test at $\alpha = 0.05$.

The WAC identifies the quantity of water absorbed by a gram of flour, without a heating process as for the WAI, and the highest WAC was shown by the F_F. This result was not previously found in studies on dry fractionation of legume flour, in which a decrease of WAC occurred in the F_F (do Carmo *et al.*, 2020; De Angelis *et al.*, 2021a,b). Probably, this unexpected result can be related to the denaturation of the proteins (Bühler *et al.*, 2020) that might have taken place during the production process of the pasta (extrusion and/or drying stage). Indeed, the heat treatment was found to enhance the capacity to absorb water in legume flour, due to the increase of the insoluble protein fraction which is the main cause of the WAC (Bühler *et al.*, 2020).

The OAC identifies the quantity of oil absorbed by a gram of flour. The C_F showed the lowest capacity to absorb oil whereas no significant differences were found between the raw material and the F_F. The decrease of the OAC in the C_F was already found by do Carmo *et al.* (2020) and De Angelis *et al.* (2021b), suggesting that this fraction could be less suitable for food applications when the incorporation of oil is needed (e.g., meat/fish products and bakery products), compared to both the raw material and the F_F (Aryee *et al.*, 2018).

The FA is related to the ability of the protein molecules to form a layer at the air–water interface, preventing the bubble coalescence (Du *et al.*, 2014; Xiong *et al.*, 2018). Therefore, the FA was significantly higher in the F_F due to the high protein content of this

fraction. The FA of the protein fraction observed in our sample agrees with the findings reported by do Carmo *et al.* (2020) on pulse protein fraction separated by dry fractionation. The foam stability at 10 and 20 min was always higher than 85%, as previously demonstrated on dry-fractionated faba bean (Vogelsang-O'Dwyer *et al.*, 2020), without significant differences between the two times. The lowest FS demonstrated by the F_F could be related to the formation of large bubbles during the homogenisation responsible for both the highest FA and the more pronounced decrease of the foam volume. The good foaming properties of the F_F suggest possible applications in dessert, mousse, and cream formulations.

Overall, despite the different trends observed after the dry fractionation process, it seems that the physicochemical and functional properties of the DLP are not far from what was assessed for the native legume flour (do Carmo *et al.*, 2020; De Angelis *et al.*, 2021a, b), highlighting that such fractions can be potentially used for the same food applications and in very similar amounts in the formulation.

Gelling behaviour

The gelling behaviour of the DLP classified with the dry fractionation is reported in Table 6. The LGC is significantly affected by the process, leading to an increment of the LGC in the C_F , and to a decrement of the LGC in the F_F , both of them by 2% compared to the raw material. The LGC indicates the gelation capacity of an ingredient. The ability to form a gel after a heating process is a peculiar property of polysaccharides, in particular the hydrocolloids – with starch mostly used as a thickening agent – (Saha & Bhattacharya, 2010), and of the proteins (Boye *et al.*, 2010; Saha & Bhattacharya, 2010). Therefore, each fraction concentration was also normalized with respect to the protein content. It is evident that a weak gel – that is, semi-solid material that flowed moderately on inversion (Boye *et al.*, 2010) – was formed when the protein concentration was in the range between 2.1 (for M_F and F_F) and 2.3 g 100 g⁻¹ (C_F). By contrast, a firm gel – that is, solid material that did not flow on inversion (Boye *et al.*, 2010) – occurred when the protein concentration was higher than 2.6 g 100 g⁻¹. Such concentrations are comparable with what was reported by Kaur *et al.* (2010) in their study concerning different species of lentils. Moreover, the LGC can be modulated by changing the pH or the ionic strength, by adding acid/bases or salts (Boye *et al.*, 2010). The study of the gelling properties is useful to identify the possible applications of the DLP and of their derived fractions for food applications, such as creams, desserts, or emulsions, that can be then produced using a low-cost ingredient with good functional properties.

Table 6 Gelling behaviour of the defected pasta before (M_F) and after separation by dry fractionation in coarse (C_F) and fine (F_F) fractions used at different concentrations

Fraction content (% w/v)	M_F	C_F	F_F
2		0.5	0.5
4		1.0	0.9
6		1.6	1.4
8	±	2.1	1.8
10	✓	<u>2.6</u>	±
12	✓	3.1	<u>2.8</u>
14	✓	3.7	✓
16	✓	4.2	✓
18	✓	4.7	✓

For each fraction is reported also the relative protein content (g 100 g⁻¹ d.m.).

: no gel formation; ±: weak gel; ✓ and underlined values: least gelling concentration; ✓: Firm gel. (Definition based on Boye *et al.*, 2010).

Conclusions

Dry fractionation can be applied to DLP, leading to the production of valuable ingredients both from a nutritional and technological point of view. Although the presence of protein–starch complexes and broken starch granules led to a lower PSE and protein content compared to the native pulse flour, the F_F showed interesting features due to the high concentration of the essential elements like Zn and Fe. Nevertheless, the relevant content of K should be considered as beneficial in the formulation of food for the hypertensive and normotensive population, while patients with impaired kidney functions may be sensitive to the high K content of the fractions. However, also the alpha-galactosides were enriched in the protein fractions, indicating that further studies are necessary to understand the influence of the food preparation on such compounds. The analysis of the functional properties highlights a good water absorption capacity and FA of the fractions, suggesting favourable utilisation in bakery products and sauces/creams/dessert preparation, further assisted by a good gelling capacity.

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Conflict of Interest

None.

Author contributions

Davide De Angelis: Conceptualization (equal); Formal analysis (equal); Investigation (equal); Writing – original draft (equal); Writing – review & editing (equal). **Antonella Pasqualone:** Resources (equal); Supervision (equal); Writing – review & editing (equal). **Luigi Manfredi:** Conceptualization (equal); Resources (equal). **Ignazio Allegretta:** Formal analysis (equal); Investigation (equal); Writing – original draft (equal). **Roberto Terzano:** Resources (equal); Writing – review & editing (equal). **Carmine Summo:** Conceptualization (equal); Formal analysis (equal); Supervision (equal); Writing – review & editing (equal).

Ethical statement

Ethics approval was not required for this research.

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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