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How to balance the yield and protein content of air-classified pulse flour: the influence of the restriction valve

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Dry fractionation by air classification is a sustainable process applied to cereals and pulses to produce protein and starch concentrates. The process involves using a series of cyclones equipped with either a classifier wheel or a restriction valve, which allow to separate a coarse starch-rich fraction and a fine protein-rich fraction. In this study, an apparatus with an air restriction valve was used, with the aim of studying the influence of two set-ups of the air classification system, on the protein content, yield, protein separation efficiency, and physicochemical and functional properties of the resulting fractions. The tighter restriction valve set-up (lower air flow and air speed compared to a more opened set-up) caused an increase in the protein content in the fine protein-rich fraction from 53.9% to 61.9%, but the drawback was a 47% yield decrease and a decrease in the protein separation efficiency. The results highlighted that the dry fractionation process should be carefully calibrated in order to balance the yield and the chemical composition (e.g. the protein content) of the fractions. In particular, the more opened set-up was better capable of balancing these two parameters, indicating that a high air flow is necessary for pulse flour. Moreover, the set-up of the restriction valve did not significantly influence effect on the physicochemical and functional properties of the fraction, pointing out that even a protein-rich fraction with a 50% protein content could be successfully used as a food ingredient.

1. Introduction

The current scenario of the food system concerns the rearrangement of the resources and of the technologies used to produce food, acknowledging the importance of sustainability and the rising consumer demand for environmentally friendly and low-processed food products (Monteiro et al. 2018). In particular, considering the protein sources, there is a spreading trend for plant-based proteins, with consequent development of innovative foods, such as meat analogues (De Angelis et al. 2020), vegetable-based beverages (Trikusuma et al., 2020), cheese analogues and dairy substitutes (Mattice and Marangoni, 2020). However, according to previous studies (van der Goot et al., 2016; Möller et al., 2021) most of the ingredients used to produce such foods are highly purified, which means, for proteins, the involvement of a considerable quantity of chemicals, and the consumption of water and electrical energy (Berghout et al., 2020), with a consequently high environmental impact. Although vegetable proteins are generally more sustainable than animal-derived proteins (Casson et al., 2019; Vogelsang-O'Dwyer et al., 2020), a concrete step forward in the development of new food systems could be achieved considering less processed ingredients (van der Goot et al., 2016; De Angelis et al., 2020). Several studies in recent years have investigated the potentiality of dry fractionation to produce sustainable protein and starch concentrates (Schutyser et al., 2015, Pelgrom et al., 2013, Pelgrom et al., 2015, Saldanha do Carmo et al., 2020; Xing et al., 2020). Dry fractionation techniques can be applied to both cereals and pulses, and the working principle of the process is based on the different size and weight of the starch granules (20-30 µm) which are heavier and larger than protein bodies (1-3 µm) (Pelgrom et al., 2013; Schutyser et al., 2015). One of the most studied methods to separate these fractions is air classification, which involves the use of a cyclone apparatus. In order to detach and split the two main components of the seeds, the dry fractionation starts with a micronisation step. Then, the flour can be separated by air classification based on both density and size (Schutyser et al., 2015). In particular, the micronised flour is fed into a cyclone and separated into two fractions by using a calibrated stream of air. The finer fraction is mainly composed of proteins (45-60%), whereas the coarser fraction is mainly composed of starch. Previous studies reported that the yield in the protein-rich fraction is inversely correlated to the protein content (Pelgrom et al., 2014, Schutyser et al., 2015), highlighting the need for a compromise to balance the yield and the protein content. To overcome this issue and to enhance the yield of separation, a system based on the combination of air classification and triboelectrostatic separation has been recently proposed (Xing et al., 2020). Therefore, the research on the dry fractionation process is still ongoing, with different equipment and technologies available on the market. For example, one technology consists of the use of a cyclone containing a classifier wheel rotating at a different speed (Pelgrom et al., 2013). Another system involves the use of the combination of a turbo separator and a cyclone, without the classifier wheel, in which the separation is only carried out by the air flow, modulated by a restriction valve (Laudadio et al., 2013). The latter does not implicate any rotating mechanism inside the apparatus, with possible advantages for the cleaning and maintenance procedures. In our study, a plant with the restriction valve technology was used, with the aim of studying the influence of two set-ups of air classification on the protein content, yield, protein separation efficiency, and physicochemical and functional properties of the resulting fractions.

2. Materials and methods

2.1 Pulse flour and dry fractionation process

Pulse flours of three different botanical species (green pea, yellow lentil, and red lentil) with the protein content of 24.8, 26.7, and 28.8% respectively (26.8% mean protein content), kindly provided by Andriani S.p.A. (Gravina in Puglia, Italy) were used for the trials. The equipment for micronisation and dry fractionation by air classification consisted of a plant of Separ Micro System sas (Flero, Brescia, Italy). Firstly, the flour was micronised to detach the starch granules from the protein bodies and the micronisation process was performed twice in a KMX-300 microniser composed of a steel drum containing a rotor moving at a peripheral speed of 170 m/s. The operating principles of the microniser were carefully described by Laudadio et al. (2013). After the micronisation, the flour was air classified in an SX-100 apparatus, consisting of a turbo separator and a cyclone. The block diagram showing the operation of the air-classifier with its input and output streams is reported in Figure 1.

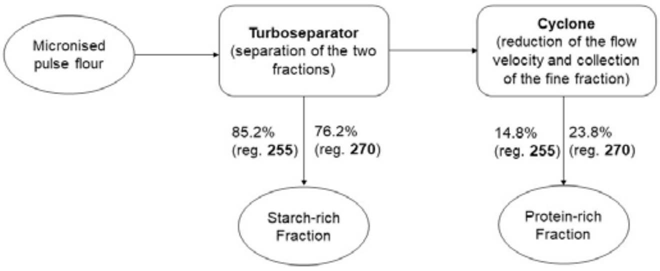

Figure 1. Block diagram of the air classification process with indication of the yield (%) of the fractions.

The turbo separator is the main part of the plant. It allows the separation of the particles composing the fraction according to their internal shape and geometry. The air flow is automatically calculated by the software of the equipment based on the flowability characteristics of the flour and it is driven by an aspirating pump installed at the end of the apparatus. However, the air flow can be modulated by regulating an inlet restriction valve, which is the only adjustable parameter during the process, and it is specific for this equipment. For this research, two different set-ups of the restriction valve were chosen on the basis of the characteristics of the flour indicated on the valve as 255 and 270, to study the relationship between yield and protein content of the fractions together with the influence on the physicochemical and functional properties. These values correspond to a tighter vs. a more opened valve set-up, respectively (the actual unit of the opening is not specified by the constructor).

The starch-rich fraction was finally collected in the turbo separator, whereas the protein-rich fraction was collected in the cyclone. The yield was calculated based on the quantity of the protein-rich and starch-rich fractions, which were divided by the total weight of the micronised flour and expressed as a percentage value.

Protein separation efficiency (PSE) was calculated as follows:

$$\mathrm{PSE} = \frac{\mathrm{P}xf \times \mathrm{Y}xf}{Pmf} \quad (1)$$

Where P*xf* is the protein content of the fraction considered (% on the dry matter), Y*xf* is the yield of the fraction considered (%), P*mf* is the protein content of the micronised flours (% on the dry matter).

2.2 Crude protein content, physicochemical and functional properties

Crude protein (total nitrogen × 6.25), was determined by Kjeldahl method, using a DKL8 Digestor and a UDK139 distillator (Velp Scientifica, srl, Usmate Velate, Italy) according to the AOAC methods 979.09 (AOAC, 2006). Moisture contents was determined according to the AOAC method 925.10 (AOAC, 2006). The analyses were carried out in triplicate. Bulk density (BD), water absorption capacity (WAC), oil absorption capacity (OAC), water absorption index (WAI), and water solubility index (WSI) of the fractions were determined according to Du et al. (2014) with the procedures described by Summo et al. (2019a). BD is expressed as g mL^{-1} and was determined by weighing the flour into a 10 mL pre-weighed cylinder. Then, the cylinder was gently tapped on the laboratory bench until no further reduction of the sample volume was observed, and the final volume was registered. WAI and WSI were analyzed by weighing in a tared centrifuge tube 1.75 g of sample and 15 mL of distilled water. The mixture was heated for 30 minutes at 70 °C and then centrifuged at 3,000 × *g* for 20 minutes. The supernatant was removed and put into a pre-weighed evaporating dish and dried overnight at 105 °C in order to calculate the solid content. At the same time, the centrifuge tube with the sediment was weighed. WAI and WSI were calculated with the following equations:

$$\mathrm{WAI} = \frac{\text{Weight of sediment in the centrifuge tube (g)}}{\text{Weight of the sample (g)}} \quad (2)$$

$$\mathrm{WSI} = \frac{\text{Weight of dissolved solids in supernatant (g)}}{\text{Weight of the sample (g)}} \times 100 \quad (3)$$

The WAC was determined by mixing 1.20 g of sample with 10 mL of distilled water in a pre-weighed centrifuge tube. The mixture was then stirred for 30 s at 5 min intervals for 30 min. The tubes were centrifuged for 20 min at 3,000 × *g*. The supernatant was discarded and the excess of water in the tube was let evaporating at 50 °C for 25 min. Finally, the sample was weighed. OAC was assessed by adding 9 mL of peanut oil to 0.75 g of sample in pre-weighed centrifuge tubes. The blend was stirred for 1 min and let rest for 30 min, the tubes were centrifuged for 20 min at 3,000 × *g*. The supernatant was discarded, and the excess of oil was removed by inclining the tubes for 25 min. Finally, the sample was weighed. WAC and OAC were expressed in g of water or oil absorbed by a g of flour.

2.3 Statistical analysis

The air classification process considering two set-ups of the restriction valve was performed on each pulse's species. The data were expressed as the mean and standard deviation of the different species and subjected to one-way ANOVA followed by Tukey's HSD (honestly significant difference) test. Significant differences were determined at *p* d 0.05 by Minitab 17 Statistical Software (Minitab, Inc., State College, PA, USA), considering the variable set-up of the restriction valve as an independent variable.

3. Results and discussion

3.1 Protein content, yield, and protein separation efficiency

Figure 1 shows the mean protein content, expressed as % on dry matter basis, the % yield, and the protein separation efficiency (PSE) of the pulse flour fractions obtained by air classification (data expressed as mean of the three species). In particular, the protein content of the protein-rich fraction significantly varied between the two different set-ups of the restriction valve. Indeed, the protein content of the fraction obtained at 255 was significantly higher than that produced by setting the restriction valve to 270, with the mean values of 61.9% and 53.9%, respectively. Interestingly, the yield of the protein-rich fraction significantly increased from 14.8% to 21.8% as the regulation of the restriction valve was more opened, with an advantageous increment of the yield by 47%. Therefore, higher protein content was associated to a lower yield. Previous studies carried out by Pelgrom et al. (2014) and Vogelsang-O'Dwyer et al. (2020) using an air classification system working with the classifier wheel reported that the yield of the protein-rich fraction is inversely correlated to the protein content. Moreover, our findings corroborate a previous study carried out by Spaggiari et al. (2020), who used the same equipment for the dry fractionation process of rice bran, studying the effect on the lipid fraction. As for the yield, also the protein separation efficiency was significantly higher in the protein-rich fraction separated

at 270 of restriction valve, with the mean value of 47.6 compared to the 34.3 of the 255 *Pf*. This suggests that the separation of the pulse flour needs high air speed and air flow, corresponding to a more opened restriction valve, to be efficient. By contrast, the protein content of the *Sf* was near 20%, without any significant differences between the two set-ups used for the restriction valve. However, when the set-up 255 was used for the air classification, the yield was higher compared to the one obtained by using the set-up 270, with the mean values of 85.2 and 76.2 respectively (11.8% increase). The results could be explained by the lower air flow and air speed occurring when the closer restriction valve was used. This determined higher retention of the micronised flour in the turbo separator, leading to a longer residence time and to the formation of sediments which, consequently, resulted in an increase in the yield. Moreover, considering the starch-rich fractions, the PSE was significantly lower in the fraction obtained with the set-up 270, suggesting a generally more profitable classification process, focused more on the efficient protein separation rather than on the mere protein content. Our results highlighted that the dry fractionation process should be carefully calibrated to balance the yield and the chemical composition (e.g. the protein content) of the fractions.
Although the 270 *Pf* was slightly poorer in protein than the 255 *Pf*, such protein concentration is still of technological interest for the food industry and it can be used for protein complementation and fortification, as well as to produce innovative food products. A very recent study demonstrated that an air classified pea protein concentrate could be used in the production of meat analogues with good functional properties and appreciated sensory characteristics (De Angelis et al., 2020). Air classified protein-rich fractions were also used for the production of baked goods by Park et al. 2014, whereas Gujska and Khan (1991) successfully used both protein-rich and starch-rich fractions to produce extruded snacks.

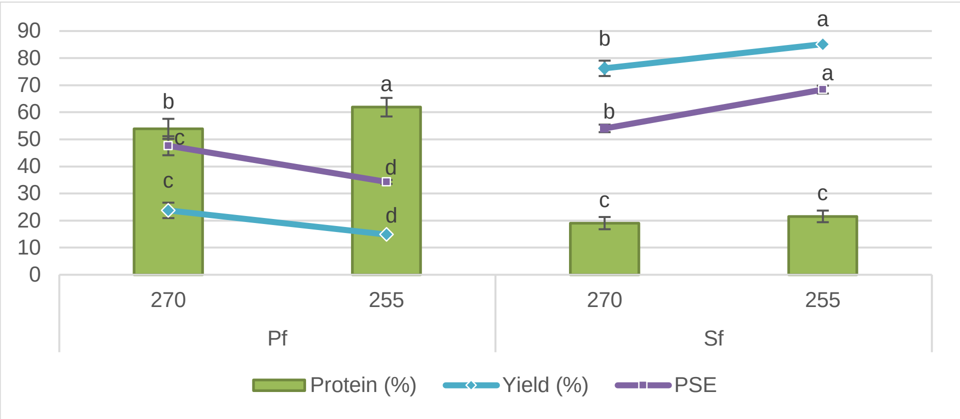

Figure 12: Protein content (% on dry matter), Yield (%), and PSE (Protein separation efficiency) of the pulse flour fractions obtained by air classification. Different letters for the same parameter mean significant differences at p d 0.05.

3.2 Physicochemical and functional properties

The physicochemical properties of the starch-rich and protein-rich fractions produced by air classification of pulse flour are reported in Table 1, together with the results of the statistical analysis with one-way ANOVA. The set-up of the restriction valve did not cause any significant effect on all the properties under investigation, highlighting that such properties are more influenced by the chemical composition (Du et al., 2014; Summo et al., 2019b; De Angelis et al., 2020). Bulk density (BD) can be defined as the mass per occupied volume and it is expressed as g/mL. The *Sf* showed significantly higher BD compared to the *Pf* and this could be explained by the coarser particle size (Drakos et al., 2017) compared to the *Pf*. Indeed, the *Sf* is predominantly composed of starch granules, whereas the *Pf* is constituted by small protein bodies, the latter leading to less dense material. The understanding of bulk density can facilitate food formulation, particularly the weaning foods (Summo et al., 2019b) in which a suitable texture is achieved by low bulk density values.
Water absorption capacity (WAC) highlights the flour's property to bind water and a slight but significant difference was found between the 255 *Sf* and both the *Pf*. Previous studies reported that the WAC is related to the presence of hydrophilic molecules, in particular dietary fiber (Du et al., 2014; Summo et al., 2019b).
Oil absorption capacity (OAC) indicates the amount of oil retained by one gram of sample. The OAC of the *Pf* was significantly higher than that of the *Sf*, displaying about the double capacity to bind oil. This result agrees with a previous study carried out by Saldanha do Carmo et al. (2020) and it can be related to the higher

presence of protein. Indeed, previously was found a significant and positive correlation between oil absorption capacity and the total protein content of pulse flour (Summo et al., 2019b).
Water absorption index (WAI) and water solubility index (WSI) are two physicochemical properties that provide information regarding the physical structure of the granules of starch and the swelling properties. In particular, WAI points out how the starch behaves after heat treatment in hot water (Du et al., 2014), whereas WSI represents the amount as a percentage of the hydrophilic compounds that remain in the water phase after the heat treatment. Despite the different chemical compositions, in our study, WAI was not significantly affected by the type of fractions, which displayed values ranging from 3.91 and 4.37, suggesting a similar swelling capacity of the starch (Du et al., 2014; Summo et al., 2019b).
Overall, the absence of significant differences in the fractions obtained by different restriction valve set-ups supports the idea that the dry fractionation process should be optimized not only for the protein content of the fractions but also on the yield. This would give better efficiency in terms of costs of this technology, leading to an easier diffusion in the food industry.

Table 1: Physicochemical and functional properties of the parent micronised flour (Mf) and the respective fractions (Sf and Pf) produced by different restriction valve settings. Different letters for the same parameter mean significant differences at p d 0.05. BD: bulk density, WAI: water absorption index, WSI: water solubility index, WAC: water absorption capacity, OAC oil absorption capacity.

Fraction	Set-up	BD g/mL	WAC g water/flour	OAC g oil/g flour	WAI g/g	WSI %
Mf		0.71 ± 0.02	0.94 ± 0.05	0.33 ± 0.02	3.96 ± 0.28	17.48 ± 4.24
Sf	270	0.89 ± 0.06[a]	0.96 ± 0.10[ab]	0.18 ± 0.02[b]	4.37 ± 0.39[a]	13.58 ± 1.02[b]
	255	0.82 ± 0.05[a]	1.00 ± 0.07[a]	0.23 ± 0.02[b]	4.31 ± 0.21[a]	15.69 ± 0.96[b]
Pf	270	0.50 ± 0.03[b]	0.80 ± 0.08[b]	0.49 ± 0.03[a]	3.91 ± 0.12[a]	24.56 ± 2.98[a]
	255	0.50 ± 0.01[b]	0.80 ± 0.05[b]	0.51 ± 0.03[a]	4.04 ± 0.10[a]	26.98 ± 1.84[a]

4. Conclusions

In this study, the dry fractionation process of pulse flours was investigated to assess the influence of two set-ups of the inlet restriction valve on the protein content, yield, protein separation efficiency, and physicochemical and functional properties of the resulting fractions. The tighter set-up of the restriction valve caused an increment of the protein content from 53.9% to 61.9%, but the yield decreased by 47%, and the protein separation efficiency decreased as well. Therefore, the 270 set-up was better capable of balancing the yield and the chemical composition (e.g. the protein content) of the fractions, suggesting that high air speed and air flow are necessary for pulse flour separation. Moreover, the set-up of the restriction valve did not cause any significant effect on the physicochemical and functional properties of the fraction, pointing out that even a *Pf* with about 50% of protein could be successfully used as a food ingredient. The results are important in order to promote dry fractionation in the food industry, and to improve the efficiency of this technology.

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