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Dry and wet fractionation of plant proteins: How a hybrid process increases yield and impacts nutritional value of faba beans proteins

Lionel Dumoulin [a, b, *], Nicolas Jacquet [a], Paul Malumba [a], Aurore Richel [b], Christophe Blecker [a]

[a] Food Science and Formulation, University of Liège – Gembloux Agro-Bio Tech, Avenue de la Faculté d'Agronomie, 2B, 5030 Gembloux, Belgium
[b] Laboratory of Biomass and Green Technologies, University of Liège - Gembloux Agro-Bio Tech, Passage des Déportés, 2, 5030 Gembloux, Belgium

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ABSTRACT

A combined dry and wet fractionation process is proposed to extract faba bean proteins with lower environmental impact. This fractionation process allowed to recover 87% of the total seeds proteins (92% of dehulled seeds proteins). This is achieved through the production of two protein concentrates (54 and 61% protein content *w*/w DM). After dry fractionation, wet extraction was performed on the protein-depleted fraction. The presented process consumed less energy and 5.5 times less water per kg extracted proteins, compared to traditional one-step wet extractions. Some anti-nutritional factors contents were also evaluated. Equivalent levels of phytic acid (about 11 mg/g), trypsin inhibitor activity (about 13 trypsin inhibition unit/g) and polyphenols (about 6 mg gallic acid equivalent/g) were observed in the 2 protein-rich fractions. These levels are mainly equivalent to those found after usual dry and wet one-step extractions. Significant differences of calcium, iron and zinc contents were observed between the 2 protein-rich fractions, causing a 30 to 50% difference between those fractions in terms of phytic acid/minerals ratio. Antinutritional factors content in the protein-rich fractions are equivalent to levels found in traditional legumes but still higher than existing recommendations.

1. Introduction

The growing demand for plant-based food products by consumers pushes the food industry to find alternatives, new feedstocks and to develop the production of functionable proteins from new plant sources (Aschemann-Witzel, Gantriis, Fraga, & Perez-Cueto, 2020). Legumes are interesting candidates to complete this challenge, regarding the high protein content, low fat content and nutritional value of their seeds (Iqbal, Khalil, Ateeq, & Sayyar Khan, 2006). When associated to cereals (containing high levels of sulfur-containing amino acids), legumes even represent a good source of all essential amino acids (FAO/WHO/UNU, 1985; Iqbal et al., 2006). Thanks to its high protein content (about 30%), faba bean is a legume of great interest for food industries (Rempel, Geng, & Zhang, 2019; Sharan et al., 2021).

One of the ways to valorize legumes is the production of food ingredients rich in proteins, such as concentrates or isolates (depending on the protein content). Extensive literature about one-step protein extraction processes applied to legumes is available. Over the years, those processes were more and more efficient and produced proteins with improved functional and nutritional properties (Coda et al., 2015; Pelgrom, Berghout, van der Goot, Boom, & Schutyser, 2014; Pelgrom, Vissers, Boom, & Schutyser, 2013; Pelgrom, Wang, Boom, & Schutyser, 2015; Rosa-Sibakov et al., 2016). However, more researches are needed to improve yield, ecological impact and costs of proteins extraction methods (Grossmann & Weiss, 2021).

Traditionally, protein extracts are recovered by wet fractionation processes, with a protein content up to 95% (*w*/w dry matter (DM)) and a protein yield of 60 to 90%, depending on the raw material (chickpea, faba bean, lupine, pea, soybean, etc.), initial protein content and process parameters (Boye, Zare, & Pletch, 2010; Fan & Sosulski, 1974; McCurdy & Knippel, 1990). Previous reviews highlighted that wet extraction processes have multiple drawbacks, such as loss of protein functional properties due to harsh extraction conditions (Assatory, Vitelli, Rajabzadeh, & Legge, 2019), or high consumption of water (about 85 kg of water/kg of protein extract (Assatory et al., 2019)) and energy (about 63 MJ/kg proteins (Assatory et al., 2019)). This could imply high costs of processing and waste management (Schutyser, Pelgrom, van der Goot, & Boom, 2015).

On the contrary, dry fractionation processes could avoid such water use and reduce energy consumption (about 5-fold reduction (Assatory

* Corresponding author at: Food Science and Formulation, University of Liège – Gembloux Agro-Bio Tech, Avenue de la Faculté d'Agronomie, 2B, 5030 Gembloux, Belgium and Laboratory of Biomass and Green Technologies, University of Liège - Gembloux Agro-Bio Tech, Passage des Déportés, 2, 5030 Gembloux, Belgium
E-mail address: lionel.dumoulin@uliege.be (L. Dumoulin).

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et al., 2019)) with equivalent protein yields (40 to 90% (Coda et al., 2015; Schutyser & van der Goot, 2011; Sosulski & Youngs, 1979)) than wet processes. Air-classification, one of the dry fractionation methods, demonstrated high possibility to reduce energy consumption (down to 11 MJ/kg of protein-rich flour) (Assatory et al., 2019). Air-classification was highly investigated in the 1970's and regained attention recently, after technological improvements (Schutyser et al., 2015).

Separation during air classification is affected by biomass hardness, its chemical composition and starch granules size (Assatory et al., 2019). Those factors explain why pulses, comprising faba bean, are good candidates for recovery of proteins by air classification. Dry-extracted proteins have better functionalities by keeping their native properties (Schutyser & van der Goot, 2011). Air classification also produces a coarse starch-rich flour (SRF), with still a relatively high protein content. Residual proteins found in the SRF are therefore a shortfall, limiting the air-classification protein yield.

More than the protein content and properties, anti-nutritional factors (ANF) contents are also of great interest for further use of protein concentrates (produced by air-classification or wet extraction, in this study) in the agro-food sector. The presence of ANF is usual in a large panel of food plants, such as cereals or legumes, leading to health issues like diminished protein digestibility, causing minerals deficiency or even presenting toxicity (Samtiya, Aluko, & Dhewa, 2020).

ANF contents are supposed to be highly dependent on wet extraction parameters such as extraction/precipitation pH's, number of washing or drying temperature after extraction. Dry extraction processes tend to concentrate ANF in protein-rich fractions due to the absence of heating and washing steps (Coda et al., 2015; Elkowicz & Sosulski, 1982; Makkar, Francis, & Becker, 2008; Xing et al., 2020). Moreover, ANF are naturally concentrated in protein bodies, by complexation to proteins (phytic acid) or as proteins (trypsin inhibition activity (TIA)). By concentrating protein bodies in the fine fraction, ANF are also concentrated in this fraction.

In this context, a new fractionation process was developed to increase protein recovery. This work presents a hybrid dry and wet fractionation process that allow to obtain protein concentrates, here with the example of faba bean seeds. This process is compared to classical dry and wet one-step extractions in terms of extraction yield, protein content and water and energy consumption. Fractions recovered along this hybrid process were also analyzed in terms of anti-trypsin activity, and polyphenols, phytic acid and minerals contents, to evaluate the main ANF contents.

2. Material and methods

2.1. Raw materials and reagents

Faba beans (FB) (*Vicia faba* L. var. fanfare, spring variety with colored flowers and high vicine-convicine content) were cultivated in Germany and sampled during the 2017 agriculture campaign. All chemicals were purchased from commercial suppliers and used as received.

2.2. Fractionation process

Hybrid fractionation process used to realize this study is shown in Fig. 1. FB seeds were first dehulled in a TM05 test Mill (Satake Corporation, Hiroshima, Japan), 15 s per run. Hulls, flour and cotyledons were further separated with a cleaner-separator-sorting device (NSP, Chopin technologies). Cotyledons and flour were collected together and dry milled below 1 mm (PULVERISETTE 19, Fritsch, Germany). Milled flour was then micronized using an Alpine grinder-classifier (Hosokawa-Alpine, Augsburg, Germany) fitted with a ZPS50 mill rotating device (8000 rpm) combined to a classifier wheel (3500 rpm), selecting particles below 40 μm (based on particle size distribution analysis, data not shown). Micronized flour was then classified with an ATP50 classifier (7750 rpm), selecting particle below 10 μm diameter (based on particle size distribution analysis, data not shown), allowing the recovery of a fine protein-rich flour (PRF) from the residual coarse starch-rich flour (SRF). This selection was repeated a second time on SRF for improved protein yield. Both PRF obtained were then mixed together to obtain a homogeneous PRF.

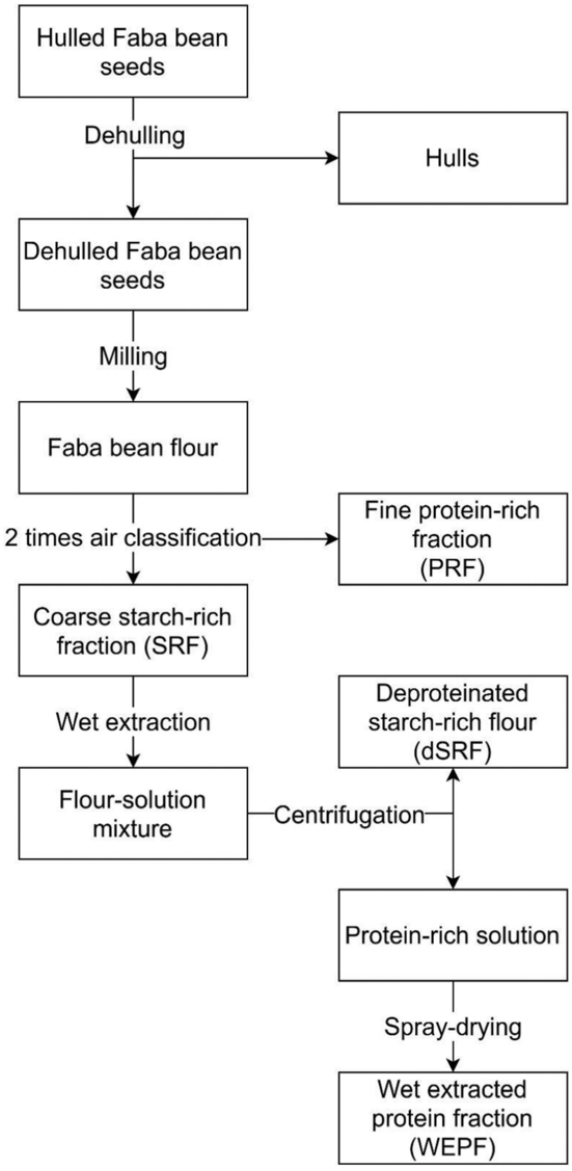

Fig. 1. Hybrid fractionation process.

Residual proteins found in the SRF were then extracted by wet extraction (60 min, pH 9.5, solid-to-liquid (S/L) ratio: 1/10). Solid and liquid fractions were separated by centrifugation (20 min, 1600*g*, 4 °C). The solid phase (deproteinized starch-rich flour (dSRF)) was washed with distilled water (S/L ratio: 1/10) and centrifuged. This was performed twice and washing water was discarded. Then, dSRF was dried at 40 °C. The protein-rich liquid phase was spray-dried (AnhydroLab S1 spray-dryer, Denmark), with an inlet air temperature of 160 °C, producing a wet-extracted protein fraction (WEPF). Air-classification parameters (milling and classifier speeds) were chosen after literature analysis and laboratory preliminary work for optimal recovery and medium protein content. Wet extraction parameters (extraction pH, time, S/L ratio, spray-dryer parameters) were selected after screening for maximal pro-

tein yield. Process was performed twice. Mass yield variability was considered negligeable.

2.3. Yield calculation

Mass and protein yields and protein shift were calculated as presented in Eqs. (1), (2), (3) (Assatory et al., 2019).

$$Mass\ Yield_i = MY_i = \frac{m_i}{m_{tot}} * 100 \quad (1)$$

$$Protein\ Yield_i = PY_i = \frac{m_i * PC_i}{m_{tot} * PC_{tot}} * 100 \quad (2)$$

$$Protein\ shift = \frac{PC_i - PC_{tot}}{PC_{tot}} * MY_i * 100 \quad (3)$$

Where m_i = dry mass of fraction i, m_{tot} = dry mass of faba bean seeds, PC_i is the protein content (w/w DM) of fraction i, PC_{tot} is the protein content (w/w DM) of the faba bean seeds.

2.4. Chemical composition

Samples dry matter (DM) was determined according to the corresponding NREL method (Sluiter et al., 2008). Protein content was estimated using Kjeldahl procedure on a Kjeltec 2300 (Foss) (Kjeldahl, 1883) with a correcting factor of 6.25 (g nitrogen/g protein). Chemical composition of each fraction is expressed as g/100 g DM. All analyses were performed in duplicates.

2.4.1. Trypsin inhibition activity (TIA)

The TIA was determined by the corresponding AACC method, as presented by Sueiro, Hermida, González, Lois, & Rodríguez-Otero, 2015, using a synthetic substrate (Nα-benzoyl-L-arginine-4-nitroanilide hydrochloride (L-BAPA)). Results are expressed as trypsin inhibition units (milligrams of inhibited pure trypsin) per mg of dry matter (TIU per mg DM). Analyses were performed in triplicates.

2.4.2. Total phenolics content (TPC)

TPC was quantified using an adapted Folin-Ciocalteu (FC) method (Singleton, 1965). After polyphenols extraction (75% aq. acetone, 60 min, 0.1 g in 15 ml), 500 µl of 10-times diluted FC reagent were added to 100 µl of sample. 2 ml of 20% (w/v) aq. Na_2CO_3 were then added to the solution. After 30 min in the dark, the absorbance was measured at 755 nm with a spectrophotometer (Shimadzu UV-1800). TPC is expressed as mg gallic acid equivalent (mg GAE) per g of DM. Analyses were performed in duplicates.

2.4.3. Phytic acid (PA)

PA content was determined by spectrophotometric quantification (Latta & Eskin, 1980). 2 g of sample were extracted by 40 ml of 2.4% (v/v) HCl (± 0.65 N) for 60 min with a rotary mixer (Labinco L27, Labinco, Netherlands). and centrifuged at 1000 g during 10 min. 5 ml of the supernatant were 10-times diluted with HCl (2,4%). 10 ml of diluted sample was eluted in a Dowex 1 × 8 200–400 mesh resin. Samples were eluted first with MilliQ water, then NaCl 0.1 M, and finally NaCl 0.7 M. The last eluted fraction was quantitatively recovered, and properly diluted with NaCl 0.7 M before mixing with Wade reagent. After centrifugation (1000*g, 10 min), the supernatant absorbance was measure at 500 nm. All Analyses were performed in triplicate.

2.4.4. Minerals content

0.5 mg of samples was digested in 5 ml regal water, before 2 h mineralization. Then, samples were solubilized in 50 ml distilled water to be analyzed through flame atomic absorption spectrometry (AAnalyst 200, Perkin Elmer, Norwalk, CT, USA) for Ca, Fe and Zn quantification. Analyses were performed in duplicates.

2.5. Statistical analysis

Data were analyzed by one-way ANOVA using Minitab Statistical Software version 19 (Minitab Inc., USA), with a significance level of $p < 0.05$. Means were compared using Tukey's test.

3. Results and discussion

In total, 87.3% of the seeds proteins were recovered in concentrated fractions. When excluding hulls proteins, 91.9% of the cotyledons proteins were recovered in either the PRF or WEPF.

3.1. Fractionation process

Figs. 2 and 3 summarizes results linked to the fractionation hybrid process. Fig. 2 presents the mass yields (A) and the protein yields (B) of the different fractions obtained at the end of the fractionation process, compared to the initial faba bean seeds, while Fig. 3 presents the protein contents of the fractions studied along the process.

As expected, results show that a majority of the mass (71%) is recovered either as PRF or dSRF. 21% of the mass is related to the hulls, which contain about 5% of the total proteins.

At the contrary, WEPF fraction (which represents only 8% of the total mass) contains 18% of the total proteins. Nearly all SRF dry mass was recovered in either the WEPF or the dSRF.

3.1.1. Seeds pre-treatments

Faba bean seeds protein content was 27.7% w/w DM. This protein content is as usually found (about 25–30%) (Alonso, Aguirre, & Marzo,

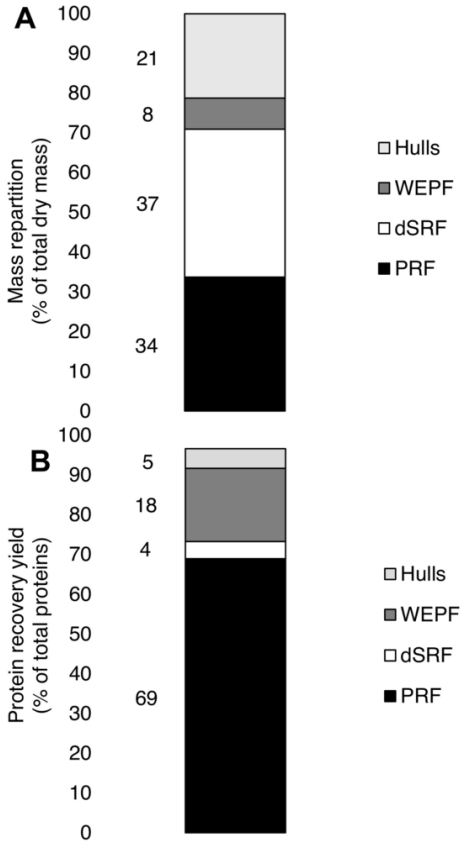

Fig. 2. A: Mass yields of the produced fraction along the fractionation process, B: Protein yields of the fractions produced along the fractionation process. WEPF = wet-extracted protein fraction, dSRF = deproteinized starch-rich flour, PRF = protein-rich flour.

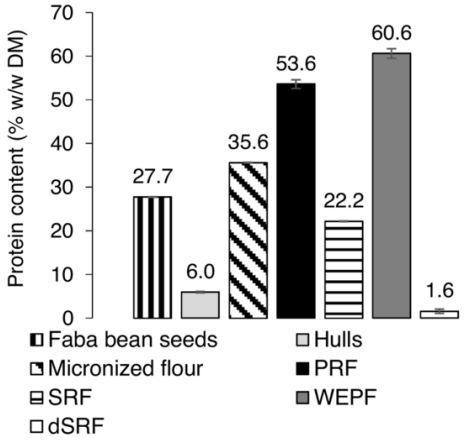

Fig. 3. Protein content of the fractions found along the fractionation process. PRF = protein-rich flour, SRF = starch-rich flour, WEPF = wet-extracted protein fraction, dSRF = deproteinized starch-rich flour,

2000; Kadam, Deshpande, & Jambhale, 1989). This initial content was first simply improved by a dehulling step, as investigated before (Saldanha do Carmo et al., 2020). After micronization, this flour has a protein content of 35.6%. Dehulling (as micronization is expected to have only little to no impact on the flour protein content) increased the protein content by 28.5%, and is characterized by a 22.5% protein shift, the highest shift observed along the presented process. This is due to the nearly total recovery of the proteins (95%) and high mass yield (79%).

3.1.2. Air-classification step

To summarize, the selected air-classification parameters allowed to recover 68.9% of the seeds proteins in the PRF, with a convenient protein content of 53.6%. A SRF with a protein content of 22.2% was also produced. The PRF protein content is consistent with values presented in the literature (50–70%) (Coda et al., 2015; Vogelsang-O'Dwyer et al., 2020). To better contextualize the air-classification step efficiency, protein bodies (subcellular organelles storing proteins (Schmidt, 2013)) have a protein content of 73% in lupine (Plant & Moore, 1983). The protein content of faba bean protein bodies was not quantified yet, to our knowledge, but are expected to be around 70%. As air-classification purpose is to separate protein bodies from starch granules, the highest possible PRF protein content is the protein content of the corresponding protein bodies (Pelgrom, Boom, & Schutyser, 2015b).

Tuning the air-classification parameters could increase the PRF protein content, compared to the results previously presented, but at the expense of the protein yield (Pelgrom et al., 2013). Moreover, the presented (high) protein recovery and (lower) protein content can be explained by the 2-times classification at the air-classification step. In this study, the main objective was the maximal recovery of proteins, justifying the double air-classification and the extraction of the remaining proteins found in the SRF by wet extraction.

The air-classification step had a protein shift of 17.0%. This parameter, evaluating the protein concentration performance of such fractionation step, is comparable to the ones published previously: 3.9 for soybean hulls, 18.2 for whole eclipse pea, 32.6 for Yellow pea and 13.2 for defatted lupine (Assatory et al., 2019; Pelgrom, Boom, & Schutyser, 2015a; Pelgrom, Wang, et al., 2015; Wolf, Sessa, Wu, & Thompson, 2002; Wu & Nichols, 2005).

As stated before, this whole process aimed to maximize the protein recovery in both protein-rich fractions. Therefore, the presented protein shift was the result of the production of a PRF with average protein content and high mass yield. Indeed, the observed protein yield (69%) is higher than found in other study (49% for yellow pea), with slightly lower protein content (53.6 compared to 57.1% (yellow pea) or 56.4% (faba bean) (Felix, Lopez-Osorio, Romero, & Guerrero, 2018; Xing et al., 2020)).

3.1.3. Wet extraction step

Wet extraction of the proteins in the SRF was performed to increase the protein yield of the whole process. The additional wet extraction was expected to produce a protein isolate (protein content >90%). However, WEPF protein content is only 60.6% (Fig. 3). This value could be explained by the lack of protein isolation (like isoelectric precipitation) and washing steps on the proteins before spray-drying between extraction and drying. The proteins were not separated from the other extracted compounds, explaining this lower protein content.

Still, this step allowed to have a protein shift of 13.5% from the SRF to the WEPF and produced an additional protein concentrate. It also produced a deproteinized starch-rich fraction (dSRF), with a protein content of 1.6%. which could enter the field of starch valorization.

3.1.4. Whole process performance analysis

In total, 87.3% of the seeds proteins (91.9% when hulls are excluded) were recovered in either the PRF or the WEPF. This is higher than generally reported in literature for wet or dry one-step and hybrid two-step legumes or Quinoa protein extraction processes (Avila Ruiz, Arts, Minor, & Schutyser, 2016; Gueguen, 1983; McCurdy & Knippel, 1990; Pelgrom et al., 2013).

To complement the analysis, water consumption was estimated. This was done by calculating the total amount of water used along the process, and dividing it by the mass of protein recovered in the protein-rich fractions (PRF and WEPF). Results showed the water consumption of the whole process is lower than traditional one-step wet extractions. As reported before, this type of extraction usually consumes about 85 kg water/kg of extracted proteins (Assatory et al., 2019). In this study, water consumption was 15.5 kg water/kg of extracted proteins (≈5.5 times lower). This amount of water could be reduced by adapting the wet extraction solid-liquid ratio, or through industrial improvement of water management. The proportional energy consumption of the whole process was also decreased compared to high-consuming wet fractionation processes (through drying) by first extract a majority of the proteins with a lower energy-consuming method (i.e. air-classification) (Assatory et al., 2019; Wang, Zhao, de Wit, Boom, & Schutyser, 2016).

3.2. Anti-nutritional factors (ANF)

Anti-nutritional factors (phytic acid, trypsin inhibition activity and polyphenolic) contents are presented in Fig. 4.

Results show that the two protein-rich fractions (PRF and WEPF) contain statistically equivalent quantities of those ANF. These fractions presented the highest concentrations of each ANF (10.8 and 10.6 mg PA/g, 14.9 and 15.6 TIU/g and 6.2 and 5.9 mg GAE/g in PRF and WEPF, respectively). ANF contents were concentrated by 137% for PA, 40% for TIA and 64% for TPC in PRF compared to micronized flour. WEPF has an ANF contents 48% (PA), 174% (TIA) and 328% (TPC) higher after extraction, compared to SRF. Increase of PA and polyphenols content from micronized flour and SRF to protein-rich fractions were expected, as they easily bound with proteins (Elkowicz & Sosulski, 1982) and are therefore extracted simultaneously, as presented before (Coda et al., 2015). Equally, as TIA is caused by specific proteins (Avilés-Gaxiola, Chuck-Hernández, & Serna Saldívar, 2018; Mekuriaw et al., 2020), concentrating proteins will also increase trypsin inhibition activity.

3.2.1. Trypsin inhibition activity

TIA in micronized flour is comparable (Guillamón et al., 2008) or slightly higher (Millar, Gallagher, Burke, McCarthy, & Barry-Ryan, 2019) than reported before. Protein extraction concentrated TIA from

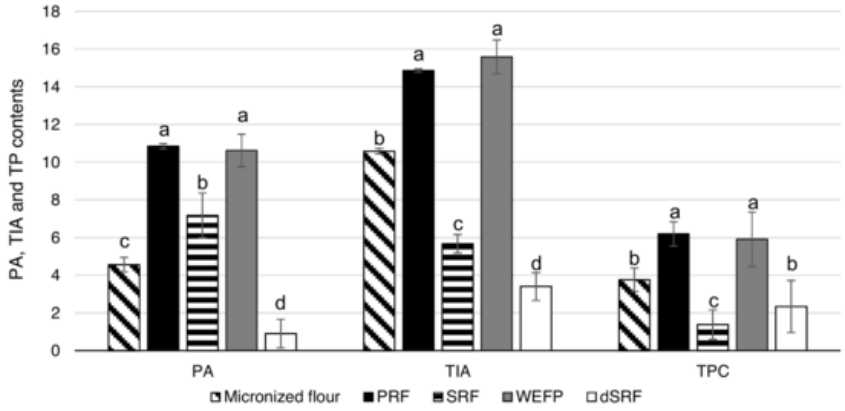

Fig. 4. Anti-nutritional factors content of the produced fractions along the fractionation process. PA = phytic acid content (mg/g), TIA = trypsin inhibition activity (TIU/g), TP = total phenolic (mg gallic acid equivalent/g). For each ANF, bars with different letters are statistically different ($\alpha < 0.05$).

the SRF to the WEPF. As stated above, this was expected as the TIA is due to specific proteins, also concentrated along the process (Elkowicz & Sosulski, 1982).

Similar TIA levels in protein-rich fractions highlights that wet extraction and drying do not allowed to totally denaturate proteins responsible of TIA. However, TIA were still lower than observed in other sources of plant proteins (before protein extraction), such as soybean (*Glycine* max, 94 TIU/mg), chickpea (*Cicer arietinum*, up to 15.7 TIU/mg) or grass pea (Lathyrus sp., 20–30 TIU/mg) (Avilés-Gaxiola, Chuck-Hernández, & Serna Saldívar, 2018; Guillamón et al., 2008). Studies on faba bean demonstrated the possibility to decrease up to 100% of TIA through supplementary treatment such as dehulling, soaking, extrusion, cooking, autoclaving, germination, fermentation and chemical processes (Avilés-Gaxiola, Chuck-Hernández, & Serna Saldívar, 2018; Mubarak, 2005).

3.2.2. Total phenolic content

Results showed that TPC levels found in produced fractions are too low to have an impact in terms of nutritional value. However, as presented before, polyphenolic compounds may have an effect on minerals bioavailability or protein digestibility. This point could only be assessed by in vivo analyses (Mekuriaw et al., 2020; Zhang, Stockmann, Ng, & Ajlouni, 2020). In previous research, cooking was demonstrated as suited to decrease polyphenolics content by up to 66% (Elsheikh, Fadul, & El Tinay, 2000).

3.2.3. Phytic acid

PA content in protein-rich fractions reach values slightly higher than found in some untreated faba bean flour, as observed in previous studies (Coda et al., 2015; Elkowicz & Sosulski, 1982). Micronized flour PA content was equivalent (Khalil, 1995) or lower (Y.-W. Luo & Xie, 2013; Millar et al., 2019) than reported before. PA content can be decreased by acidic soaking and cooking (−35%), dry-heating (−36%) or germination (−45%) (Vidal-Valverde et al., 1998).

PA equivalent contents in protein-rich fractions is not a proper indicator of equivalent effect on nutritional value. Previous reports of FAO/WHO indicate that PA content must be compared to Fe, Ca and Zn contents for proper evaluation of the impact of PA chelation of these minerals, and so to estimate their biodisponibility to be assimilated. Acceptable ratios (mol PA/mol mineral, as reporter by the WHO/FAO) are below 1 for iron (Fe^{2+}) (or ideally 0.4), 0.24 to 0.17 for calcium and between 5 and 15 for zinc (Roos et al., 2013; Zhang et al., 2020).

Ratios in Table 1 show that micronized flour was acceptable for direct consumption in terms of minerals biodisponibility, except iron-wise. Unfavorable PA/Fe ratios are commonly found in legumes, such as in field pea (PA/Fe of 3.79), soybean (8.02) or lentils (3.9) (Sandberg, 2002).

Table 1
Molar ratio phytic acid/mineral of the fraction produced along the process, SRF = starch-rich flour, PRF = protein-rich flour, WEPF = wet-extracted protein fraction, dSRF = deproteinized starch-rich flour. Bold numbers are considered unacceptable when confronted to WHO/FAO recommendations.

	PA/Ca	PA/Fe	PA/Zn
Micronized Flour	0.12	**3.7**	4.4
SRF	**0.43**	**8.1**	**9.5**
PRF	**0.42**	**9.4**	**11.0**
WEPF	**0.29**	**5.0**	**5.8**
dSRF	0.03	**1.3**	1.5

Although PA contents are comparable between the 2 protein-rich fractions, the estimated biodisponibility of Ca, Fe and Zn aren't. Mineral contents in PRF are too low to allow proper assimilation of Iron and Calcium, or Zinc. However, WEPF minerals biodisponibility is sensitively higher. PA/Ca is 31% lower than in PRF, at a level near the limits (0.29, compared to a limit of 0.24). A decrease of 47% of the PA/Fe ratio was not sufficient to be under the FAO/WHO recommendations (ratio of 1).

With equivalent PA contents, those results are only explained by differing minerals contents. Additional minerals contained in the WEPF could be explained by their high solubilization during the liquid extraction. As the proteins were not washed, minerals were concentrated during the spray-drying. As a result, 37% of Ca, 50% of Fe and 41% of Zn found in the SRF are found in the WEPF, while only 26% of the SRF PA were extracted. This led to proteins containing less PA than after a doubled air-classification step. These observations can be used to choose a proper treatments (enzymatic, thermal treatments, or even fermentation) to reach acceptable PA contents in both protein-rich fractions (Y.-W. Luo & Xie, 2013; Zhang et al., 2020)).

The quantification of Ca, Fe and Zn in dSRF showed that 0%, 37% and 25% of those minerals were lost during the SRF washing before drying, possibly due to differential affinity to proteins and/or PA. Up to 64% of PA was lost. Differential solubility of minerals and PA for improved PA/mineral ratios is to be further studied. With appropriate phytic acid elimination or denaturation (through enzymatic treatment, for example), this aqueous effluent could be a source of minerals to reinject in the process to increase the nutritional value of the produced protein fractions. Evaluation of the chemicals and energetic cost has to be evaluated before considering adding such treatment in the proposed process.

Recent study highlighted the limitations of studying phytic acid-minerals ratio to evaluate minerals biodisponibility (Zhang et al., 2020). Various interference factors were presented, such as proteins properties, due to their 3D conformation. However, as presented by the authors, traditional evaluation of molar ratio stays valid in some cir-

cumstances and is still an easy and quick method to do a first evaluation of minerals biodisponibility.

Biofortification through proper soil fertilization and the use of biotechnological tools are currently evaluated to fight Fe and Zn nutritional deficiency in human food. Those methods would be used to increase Fe and Zn accumulation in faba bean cotyledons or their biodisponibility. Also, studies demonstrated the possibility to decrease ANF absolute content through varieties selection, breeding or post-harvest treatments (thermal, chemical, enzymatic or biological) (Avilés-Gaxiola et al., 2018; Coda et al., 2015; Helsper, Hoogendijk, van Norel, & Burger-Meyer, 1993; Khazaei et al., 2019; Y. Luo, Xie, & Cui, 2010; Mubarak, 2005; Rehman & Shah, 2005; Sánchez-Chino, Jiménez-Martínez, Dávila-Ortiz, Álvarez-González, & Madrigal-Bujaidar, 2015). However, such methods are water and energy consuming, and would be added to already existing processes, increasing their environmental impact. This increases the need to develop fractionation and treatment processes with minimal water and energy consumption. No regulation on ANF contents exists, and the only recommendations are coming from the FAO and WHO on phytic acid-mineral ratios. This leads to the impossibility of giving a proper answer to the question: "Are those protein-rich fractions at least not harmful?" due to lack of objective factors.

4. Conclusion

In this study, a new dry-wet fractionation process, allowing the recovering of 87% of faba bean seeds proteins (92% of dehulled seeds proteins) in concentrated fractions, was developed. This was achieved with 5.5 times decrease of water use and theoretical decrease of energy consumption. It allowed to produce protein concentrates with convenient protein contents (54 and 61% *w*/w DM). At the end of the process, a starch-rich fraction containing only 1.6% proteins was produced. This fraction would be available for further starch valorization and so increase the presented process profitability.

Besides process analysis, ANF contents of the produced proteins were evaluated. It was observed that ANF contents increased after protein extraction steps, between 40 and 330%, depending on the ANF and the protein extraction step. However, the contents/activity observed in the protein-rich fractions are still below those observed in some unprocessed legumes. It can only be stated that the 2 produced fractions have potentially more adverse effects on human health than micronized dehulled faba bean flour, in a statistically equal way. Only the PA/minerals ratios allowed a differentiation of PRF and WEPF nutritional values, with more favorable ratios in WEPF.

Further study should be made on other ANF, such as vicine-convicine, lectins, saponins contents or other inhibition activities, to have a better vision of the nutritional value of the produced fractions.

CRediT authorship contribution statement

Lionel Dumoulin: Conceptualization, Methodology, Formal analysis, Writing - original draft. **Nicolas Jacquet:** Conceptualization, Writing - review & editing, Funding acquisition. **Paul Malumba:** Conceptualization, Writing - review & editing, Funding acquisition. **Aurore Richel:** Writing - review & editing, Funding acquisition. **Christophe Blecker:** Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

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