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# Production of proteins and phenolic compounds enriched fractions from rapeseed and sunflower meals by dry fractionation processes

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ABSTRACT

Rapeseed (RSM) and sunflower (SFM) meals are highly abundant and protein-rich by-products from the oilseed industry. Besides their basic use as animal feed, they are seen nowadays as interesting raw materials for the production of high value added products such as protein isolates, peptides, emulsifiers and biomaterials. In other respects, they contain significant amounts of phenolic compounds exhibiting antioxidant or antimicrobial properties but widely untapped so far. Therefore, any process allowing the single-step separation of both the protein and phenolic parts of meals would be beneficial to the whole oilseed sector. To achieve this double objective this study attempted to separate the RSM and the SFM into their major constituents by using dry fractionation technologies. In a first step, ultrafine milling was applied to the meals. As a function of raw material type, the grid size turned out to be decisive on the particle size distribution and its modality. Then two separation technologies based either on particle charge (electrostatic sorting – ES) or density (turbo-separation – TS) were applied to the previously obtained fractions. Regardless the separation technique, the best results were obtained from fractions of an average particle diameter by mass ($D_{50}$) of 23.7 ± 1.0 µm and 105.5 ± 8.3 µm, for RSM and SFM respectively. Electrostatic sorting allowed increasing simultaneously the protein and phenolic contents by 50–55% and 80–100% for RSM and SFM respectively, while a lower increase was observed for turbo-separation (23–29% and 58–64% for RSM and SFM respectively). Finally, depending on the process and meal types, the overall recovery yield of the most enriched fractions was in the range of 30–40%.

## 1. Introduction

Rapeseed and sunflower are the most important oil crops in Europe (Carré and Pouzet, 2014). Their oils are considered as one of the healthiest due to their high content in mono- and poly-unsaturated fatty acids and tocopherols (Luigicioni, 2005; Shahidi, 1990). Rapeseed meals (RSM) and sunflower meals (SFM) are co-products of the pressing and de-oiling process of their seeds. In 2016, the production of RSM and SFM in France were estimated to be 2.6 Mt and 0.6 Mt respectively (Terres Univia 2016). RSM and SFM are heterogeneous materials that contain proteins (36–38 g/100 g DM) (28–30 g/100 g DM), lignin (9–11 g/100 g DM) (11–13 g/100 g DM), cellulose (13–15 g/100 g DM) (25–27 g/100 g DM) and phenolic compounds (≈2 g/100 g DM) (≈ 4 g/100 g DM). For both RSM and SFM, proteins and phenolic compounds are mainly present in the kernel (Carré et al., 2016; González-Pérez and Vereijken, 2007; Weisz et al., 2009) while lignin, cellulose, and hemicellulose are more concentrated in the hull (Cancalon, 1971; Carré et al., 2016). If meals are currently mostly intended for cattle feed, oleaginous proteins, especially from soybean, canola or sunflower, may be transformed into high added-value products such as bio-packaging or emulsifiers (Zhang and Mittal, 2010; Shi and Dumont, 2014) but also adhesives and fiberboards (Wang et al., 2014; Evon et al., 2015). Moreover, several studies have demonstrated the antioxidant activity of peptides from rapeseed meals protein

*Abbreviations:* CAE, chlorogenic acid equivalent; CF, coarse fraction recovered after turbo-separation; DM, dry matter; DDM, delipidated dry matter; $D_{50}$, mass-median-diameter of particles (average particle diameter by mass); ES, electrostatic sorting; FF, fine fraction recovered after turbo-separation; NFc, negatively charged particles collected on the corresponding jar; NFe, negatively charged particles collected on the positive electrode; PFc, positively charged particles collected on the corresponding jar; PFe, positively charged particles collected on the negative electrode; RSM, rapeseed meal; SAE, Sinapic acid equivalent; SFM, sunflower meal; TPC, total phenolic compounds; TS, turbo-separation; UFM, ultrafine milling

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hydrolysates (Aider and Barbana, 2011; Zhang et al., 2008), while proteins associated with phenolics can act as emulsifier displaying antioxidant activities (Guimarães Drummond e Silva et al., 2017; Rawel et al., 2005).

Besides, naturally occurring phenolic compounds are known to be bioactive molecules exhibiting antioxidant (Dimitrios, 2006), anti-inflammatory (Hwang et al., 2014) and antimicrobial activities (Xu et al., 2016) to name a few. This is why they are already widely used as preservatives in the agri-food and cosmetic industries for the protection of unsaturated lipid-containing systems and enhancement of their shelf life (Durand et al., 2015; Shahidi and Ambigaipalan, 2015).

Even today, it remains a challenge to separate the proteins from the other constituents of RSM and SFM, i.e lignin, phenolics, carbohydrates. To do this, different methodologies have been developed such as solid-liquid extractions (Aider and Barbana, 2011; Das Purkayastha et al., 2013; Kachrimanidou et al., 2015). Unfortunately, these routes not only generated effluents but were also ineffective to individually segregate meal constituents.

As a promising alternative to solvent-assisted processes, dry fractionation technologies have been recently developed and successfully applied to various raw materials including oleaginous meals. These dry fractionation processes can be divided into two steps. First, the milling step in which the different constituents of the meals are detached from the cellular matrix. Then, the separation step which is based on the differences in the physicochemical properties of the different constituents. Recently, ultrafine milling (UFM) coupled to electrostatic sorting (ES) or turbo separation (TS) emerged as eco-friendly technologies suitable for the concentration of proteins, cellulose, lignin and polyphenols from many agro-resources (Chuetor et al., 2015; Hemery et al., 2011; Pelgrom et al., 2014). Moreover, these processes are significantly more energy efficient than solid-liquid fractionation processes and are able to produce enriched fractions with retained (native) functionality.

Although ES of fractionated RSM and SFM has already been implemented (Barakat et al., 2015; Basset et al., 2016), the influence of the particle size distribution on the separation steps was not studied. Plus, a fine characterization of the different phenolic compounds of the different fractions was not fully done. In addition, to the best of our knowledge, the TS of the RSM and SFM have never been studied. Knowing this, the aim of this study was to compare the dry fractionation processes of RSM and SFM by applying UFM coupled to ES and TS technologies.

In this work, the study of the UFM conditions and the influence of the particle size distribution on the separation steps were of paramount importance. Here, shear and impact milling were applied to the RSM and SFM. To obtain ultra-fine meals with different particle size distributions, three different grid size were used. Then, the three ultra-fine meals were separated by a single step ES or TS to investigate the impact of the milling conditions. To know whether the separation was effective, the chemical composition (protein, lignin, individual and total phenolic compounds (TPC) contents) and the particle size distribution of the different fractions were determined. After establishing the best milling conditions, a methodology to increase the recovery yield of the fractions of interest during ES was developed.

## 2. Materials and methods

### 2.1. Chemicals

Sinapine thiocyanate (99.0% HPLC) was isolated from a methanolic extract of rapeseed meal according to the method outlined by Mailer *et. al* (Mailer, 2008). 3,4-; 3,5- and 4,5-di-O-caffeoylquinic acids (> 99.0% HPLC) were isolated from yerba maté leaves by the method outlined by Tong *et. al* (Tong et al., 2015). Sinapic acid (98%) was from AlfaAesar (Karlsruhe, Germany). 5-caffeoyl quinic (Chlorogenic) acid (95%), caffeic acid (> 98%), methanol and water (for HPLC, > 99.9%) were purchased from Sigma–Aldrich (Saint-Quentin-Fallavier, France).

### 2.2. Raw materials

Experiments were carried out on rapeseed (RSM) and sunflower (SFM) meals prepared at the pilot oil plant of OLEAD (Pessac, France), according to the following methods.

#### 2.2.1. Preparation of the rapeseed meal (RSM)

Whole rapeseeds (120 kg) were first cold pressed on a MBU20 screw press (OLEXA, France), fed at 75 kg/h of seeds, to remove 75% of the initial oil amount. The residual oil of the press cake was further extracted by steeping in hexane at 50 °C, in a Guedu Pilot Agitated Filter Dryer (De Dietrich Process Systems) of 480 L total capacity. Extraction was performed on 73 kg of press cake by immersion in hexane for 15 min followed by filtration. Five successive steps of immersion-filtration were required to remove oil from the cake. The defatted cake was then desolventized in the same device, under vacuum at 60 °C and without injection of steam. The water and lipid content of the resulting RSM were of 11 ± 0.0% and 1.7 ± 0.1% respectively.

#### 2.2.2. Preparation of the sunflower meal (SFM)

Whole sunflower seeds (75 kg) were first cold flaked in a flaker (Damman-Croes N.V., Belgium) equipped of two contra-rotating smooth cylinders of 500 mm in diameter, spaced of 0.3 mm (capacity: 200 kg/h). The flakes (73 kg) were then deoiled by hexane-extraction at 50 °C in a Guedu Pilot Agitated Filter Dryer (De Dietrich Process Systems) for 15 min, followed by filtration. The immersion-filtration step was repeated six times. The defatted cake was finally desolventized in the same device, under vacuum at 60 °C and without injection of steam. Finally, sunflower meal were coarsely milled with a knife milling using a 2 mm gird The water and lipid content of the resulting SFM were of 7.5 ± 0.0 and 2.0 ± 0.1% respectively.

### 2.3. Ultrafine milling

Raw RSM and SFM were previously coarsely crushed with a Retsch SM 300 knife mill (Retsch Technology GmbH, Haan, Germany) operating at 2000 rpm with a two millimeters grid. Then, the samples were finely ground by impact and shear mill UPZ100 (Hosokawa-alpine, Augsburg, Germany) using different grids of 0.5, 0.3 and 0.1 mm (0.2 mm for the SFM). The device was operated at room temperature, at a speed of 18 000 rpm, and a feeder speed of 4 kg $h^{-1}$. The material was milled until it passed through the grid. The particle size distribution of the samples was determined by laser diffraction using a Mastersizer 2000 in combination with the Scirocco 2000 dry dispersion unit (Malvern Instruments, Worcestershire, UK). All measurements were performed in triplicate.

### 2.4. Electrostatic sorting

A pilot electrostatic separator (TEP System, Tribo Flow Separations, Lexington, USA) was used for the fractionation of the different ultrafine RSM and SFM. The feeding system of the separator was operated at 1.2 kg/h with an initial amount of meal of 250 g; the particles were then conveyed by compressed air (gas flow rate = 5.1 $m^3$ $h^{-1}$) in a teflon tube (250 cm, $Ø_{in}$ = 11 mm, $Ø_{ext}$ = 13 mm) where they were charged by triboelectricity, i.e. by impacting each other and against the walls of the charging line. Finally, charged particles passed through a separation chamber containing two high voltage electrodes (7.5 cm x 28.0 cm; 10 000 V), where the positively charged particles are attracted by the negative electrode and the negatively charged particles are attracted by the positive electrode. A particle recovery system equipped with two collecting jars allowed to separately recover two fractions: one containing the positively charged particles named the collected positive fraction “PFc” and the other, the negatively charged particles named

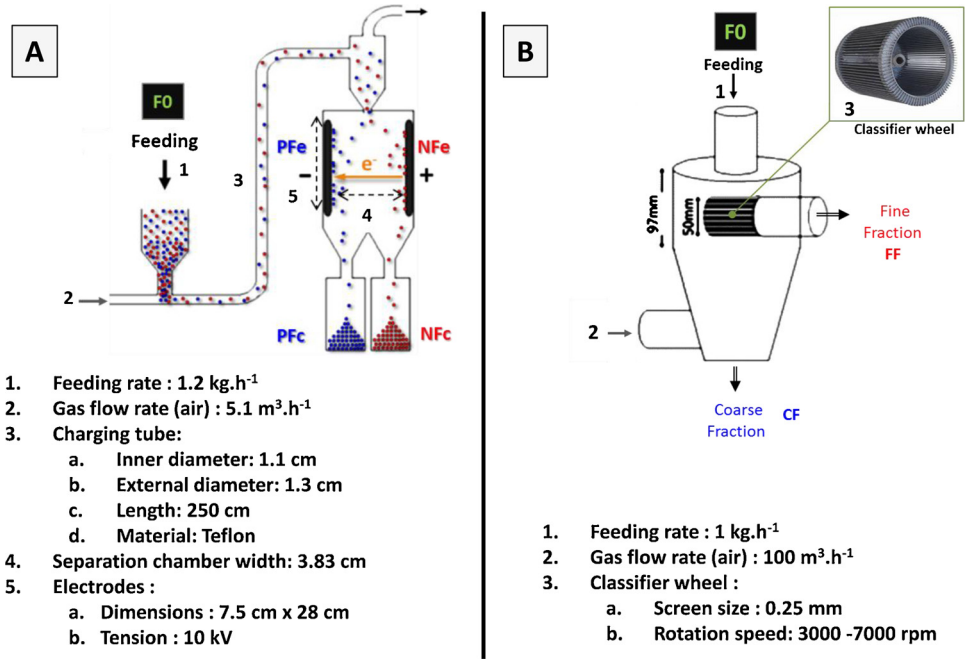


**Diagram 1.** Device and operating conditions implemented for electrostatic sorting (A) and turbo-separation (B).

the collected negative fraction "NFc". In addition, the particles agglomerated on the electrodes were manually recovered and were considered as the purest fractions: the negative electrode containing the positively charged particles named the positive fraction of the electrode "PFe", and the negatively charged particles named the negative fraction of the electrode "NFe". To avoid the saturation of electrodes, particles were recovered around every 2 min. The four different fractions obtained were subjected to chemical and physicochemical analyses. Electrostatic sorting device and operating conditions are described in Diagram 1A. All experiments were performed in triplicate.

Recycling steps applied to RSM-0.1 and SFM-0.5: after each separation, the particles agglomerated on the electrodes were recovered separately, and the two fractions collected in jars were mixed and subjected again to separation. This step was repeated until no powder could be recovered on the electrodes. The number of recycling step depends on several factors among which, the initial sample amount and composition, the electrode dimensions (the larger the surface the higher the amount recovered at each recycling step) and, in a lesser extent, the design of the charging line and the flow rate of particles inside it. Under the experimental conditions settled above, four and three recycling steps were respectively needed for RSM-0.1 and SFM-0.5 samples.

### 2.5. Turbo-separation

A turbo-separation (or air classification) pilot equipment (Hosokawa alpine, Japan), was used to produce coarse fractions (CF) and fine fractions (FF) displaying different densities. The core element of the fine sizing plant is the 50 ATP Turbo-separators. The instrument was use as described by Chuetor et al., 2015. Briefly, the particle feeding was operated at 1 kg/h with an initial amount of meal of 250 g; the gas flow rate (air) was set at 100 $m^3 h^{-1}$ and the rotation speed of the classifier wheel (screen size = 0.25 mm) varying from 3000 to 7000 rpm. The different fractions obtained were subjected to different chemical and physicochemical analysis. Turbo-separation apparatus and operating conditions are described in Diagram 1B. All experiments and measurements were performed in triplicate.

### 2.6. Dry matter, ash, and protein analyses

The dry matter, ash and protein contents of the different fractions were determined according to AACC-approved methods 44–19, 08–12, and 46–12 (with N × 6.25), respectively. All measurements were performed in triplicate.

### 2.7. Oil content

Around two grams of each fraction were placed into a filter paper envelope previously dried and weighed and the whole was dried at 105 °C until constant weight. Then, envelopes were extracted with n-hexane for 5 h using a Soxhlet apparatus. After extraction, envelopes were desolventized at room temperature for 12 h, dried at 105 °C and finally weighed. The oil content was calculated from the difference between the dry weight of the sample (meal and envelope) before and after delipidation. Values were given as percentage of oil per gram of dry matter. Each determination was done in triplicate.

### 2.8. Extraction of phenolic compounds

The extraction of phenolic compounds of each fraction obtained after the dry fractionation processes was carried out following the procedure described by Cai and Arntfield (2001) using a 100/1 solvent/sample ratio. Typically, 50 mg of the sample were weighed into a brown flask with a screw cap and 5 mL of methanol were added. Closed flasks were incubated in an orbital shaker (350 rpm) at 75 °C for 20 min, then cooled to room temperature and centrifuged 5 min at 4000 rpm. The methanol phase was collected and evaporated under a nitrogen stream. The dried extracts were kept at −20 °C until analysis. In the above mentioned conditions, a single step extraction allows the full recovery of phenolics (> 99%). All experiments were done in triplicate.

### 2.9. Determination of individual and total phenolic contents by HPLC

The sample dry extracts previously obtained were solubilized in 5 mL methanol/water 2/1, v/v) and filtered (0.45 µm nylon filters) before HPLC analysis. Phenolic compounds were quantified with an XR UFLC Shimadzu liquid phase chromatograph equipped with an SPD-

M20A diode array detector (Kyoto, Japan). Separation was carried out with an ACE C18 reversed phase column (5 µm, 250 mm × 4.6 mm, Phenomenex, Le Pecq, France). Injection volume, flow rate, and oven temperature were set at 20 µL, 1 mL/min and 30 °C respectively. The gradient elution was performed using HPLC grade water with 0.1% (v/v) acetic acid (A) and methanol with 0.1% (v/v) acetic acid (B) in the following conditions: 15% B (0–5 min), 15–80% B (5–30 min), 80–100% B (30–31 min), 100% B (31–35 min), 100-15% B (35–40 min).

#### 2.9.1. Rapeseed samples

Sinapic acid, and sinapine thiocyanate were chosen as external standard for calibration curves. Their maximum wavelength of detection and retention time were 323 nm–17.8 min and 328 nm–11.5 min respectively. The total phenolic content was calculated from the total area of sinapic acid and all its esters at 328 nm and expressed in sinapic acid equivalent (SAE)/g DDM. Calibration curves were obtained from series of standard methanolic solutions from 12.5 to 200 µmol $L^{-1}$ with $R^2$ = 0.9987 for sinapine and $R^2$ = 0.9983 for sinapic acid. All experiments were done in triplicate.

#### 2.9.2. Sunflower samples

The maximum wavelengths of detection were 323 nm for caffeic acid, 326 nm for 3-, 4- and 5-caffeoylquinic acids, 328 nm for 3,5-and 4,5-di-caffeoylquinic acid and 326 nm for 3,4-di-caffeoylquinic acid. The retention times were 12.1 min for 5- and 4-caffeoylquinic acid (co-eluted), 17.8 min for 3,5-di-caffeoylquinic acid and 21.3 min for 4,5-di-caffeoylquinic acid. The total phenolic content was calculated from the total area of caffeic acid and all its esters at 326 nm and expressed in chlorogenic acid equivalent (CAE)/g DDM. 5-caffeoylquinic acid was selected as external standard and the calibration curve was obtained from series of standard methanolic solutions from 12.5 to 800 µmol $L^{-1}$ ($R^2$ = 0.9991). All experiments were done in triplicate.

### 2.10. LC–MS characterization of main phenolic compounds in rapeseed and sunflower meal extracts

RSM and SFM methanolic extracts were analyzed by LC–MS using a UPLC Acquity H-Class (Waters) equipped with a diode array detector. Separation was carried out with a Kinetex C18 100A 100 × 2.1, 2.6 µm (Phenomenex). Injection volume, flow rate, and oven temperature were set at 1 µL, 0.5 mL/min and 25 °C respectively. The gradient elution was performed using HPLC grade water with 0.1% (v/v) formic acid (A) and methanol with 0.1% (v/v) formic acid (B) in the same condition as in Section 2.8.

The mass spectrometry detection was done with a Synapt G2-S (Waters) equipped with an ESI source. The capillary tension and the cone tension were of 3 000 V and 30 V respectively. The source temperature and the desolvation temperature were of 140 °C and 450 °C respectively. The analysis was carried out in scan mode (100–1500 Da) using both positive and negative ion mode.

### 2.11. Carbohydrates analysis

The carbohydrate and lignin composition of lignocellulose samples were measured after acid hydrolysis. The lignin content in samples was determined by the Klason method. Briefly, 80 mg of each fraction were hydrolyzed with 72% sulfuric acid for 1 h at 30 °C. The solutions were diluted with water to 4% $H_2SO_4$ and heated at 120 °C for 65 min. The hydrolysates were filtered (10 µm) and the Klason lignin content was determined as the weight of the residue retained on the filter after drying at 105 °C for 24 h. All determinations were carried out in triplicate.

### 2.12. Scanning electron microscopy

Scanning electron microscopy SEM (Phenom G2 Pure apparatus, Phenom-World BV, Eindhoven, Netherlands) was used to observe the ultra-fine RSM and SFM fractions. The samples were set on a double-sided adhesive tape and fixed to SEM stubs. Excess particles were removed with a dry air stream. Pretreatment of the samples was not necessary.

### 2.13. Statistical analyses

Means and SD were calculated using Minitab Statistical Software v.18. The means were then compared using a one-way ANOVA analysis. The significance level α of statistical analysis was set to 0.05.

## 3. Results & discussion

### 3.1. Individual phenolic compounds of rapeseed and sunflower meals

Studies have shown that in RSM, the concentration of phenolic compounds can be as high as 2% DM. Sinapine, the choline ester of sinapic acid, accounts alone over 80% of all the phenolic compounds, the rest being almost all esterified sinapic acid with sugars and kaempferol (Baumert et al., 2005). In SFM, the total phenolic content can be as high as 4% DM. Chlorogenic acid (5-caffeoylquinic acid; 5-CQA) represents around 70% of the total phenolic compounds while the others 30% are chlorogenic acid isomers and di-caffeoylquinic acids (Weisz et al., 2009).

In this study, the ultra-fine RSM and SFM obtained with the 0.1 mm grid (named RSM-0.1) and 0.5 mm grid (named SFM-0.5) were respectively selected as reference materials. Phenolic compounds were fully extracted with pure methanol at 75 °C, structurally identified by HPLC–MS and quantified by HPLC-DAD. In the RSM-0.1, nineteen different phenolic compounds were found (Fig. 1A, Table 1). As expected, the main compound was sinapine, with 12.4 ± 0.2 mg/g DDM, accounting over 75% of all phenolics, while the free trans-sinapic acid content was only 0.9 ± 0.0 mg/g DDM. The other phenolic compounds, whose structure was elucidated by HPLC–MS and listed in Table 1, were identified as sinapic acid esters comprising a sugar (glucose, gentobiose) and/or a kaempferol moiety. These results were in good agreement with literature data (Baumert et al., 2005; Siger et al., 2013; Zago et al., 2015). Regarding the TPC content, the Folin-Ciocalteu method is usually considered as the reference since only a few of phenolic compounds are commercially available and used as external standard for calibration. However, this simple and fast methodology is not accurate, nor specific, since all reducing species (e.g. sugars, small peptides) (Peterson, 1979; Vuorela et al., 2004) are likely to react with the Folin-Ciocalteu reagent, thus leading to overestimated values. Here, the TPC content was calculated from HPLC data obtained at 328 nm (corresponds to $\lambda_{max}$ of sinapine, the major phenolic compound), by summing peak area of all sinapoyl-containing molecules. An average TPC content of 16.6 ± 0.3 mg of sinapic acid equivalent (SAE)/g DDM was found for RSM-0.1, which is in the range of values reported so far (Cai and Arntfield, 2001; Khattab et al., 2010).

For SFM-0.5, eleven different phenolic compounds were identified, the main species being chlorogenic acid (5-caffeoylquinic acid, 5-CQA) with 17.3 ± 0.3 mg/g DDM. Besides, caffeic, 3-caffeoylquinic, mono- and di-caffeoylquinic acids were also identified in the methanolic extract (Fig. 1B, Table 2) from the retention times of authentic standards or literature data (Weisz et al., 2009). For RSM-0.5, the TPC content of 25.3 ± 0.4 mg chlorogenic acid equivalent (CAE)/g DDM was in agreement with other published results (Pedrosa et al., 2000; Weisz et al., 2009). This value was calculated from HPLC data, in the same way as for rapeseed meal.

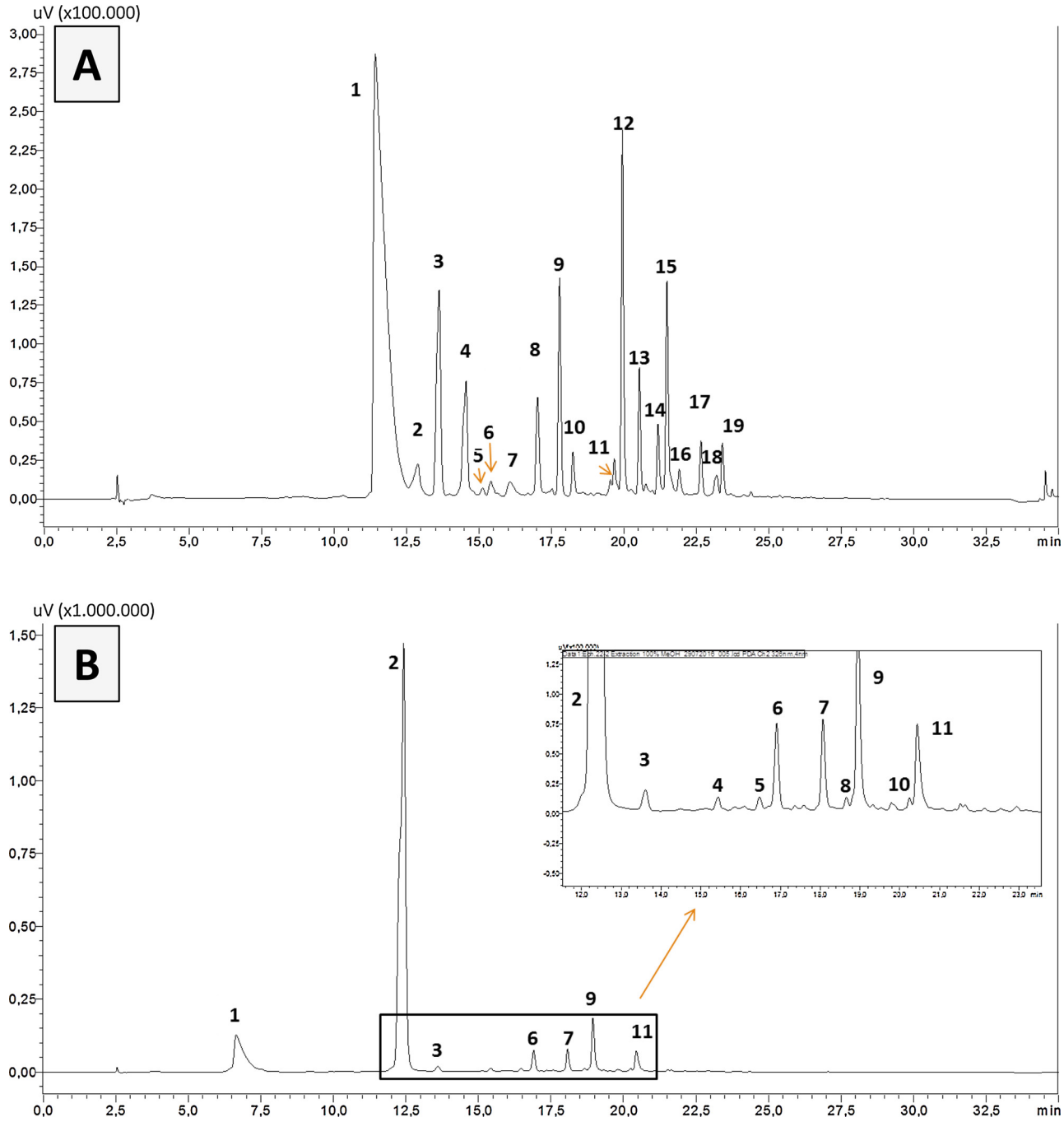


**Fig. 1.** HPLC profile of RSM-0.1 (A) and SFM-0.5 (B) methanolic extracts at 328 nm and 326 nm respectively.

### *3.2. Effect of ultrafine milling on meal characteristics*

Before the UFM step, raw RSM and SFM were coarsely milled with a knife milling using a 2 mm grid. Then, an impact and shear milling was applied using a UPZ100 Fine Impact apparatus. To obtain different particle size distributions, three different grid sizes were used: 0.5 mm, 0.3 mm and 0.1 mm (0.2 mm for the SFM). For the rest of this study, the different ultra-fine meals were named RSM-"size of the grid" and SFM-"size of the grid". As expected, decreasing the grid size led to the proportional decrease of the $D_{50}$ of each meal (Table 3). This decrease had no significant effect on the recovery yield (around 96% w/w) except for SFM-0.2 (88.8 ± 0.1% w/w). In the same way, the content in individual phenolics (sinapine or 5-CQA) and TPC were not affected in both meals. On the other hand, the reduction of the grid size was accompanied by a significant increase in the protein content, particularly noticeable in case of SFM-0.2. This might be explained by the difference in the physical and mechanical properties of rapeseed hulls that are more resistant and hard to mill than sunflower ones (Asad et al., 2017). Indeed, when all the particles including fiber-rich ones (mostly the hulls) are milled down to very small sizes, this resulted in the loss of the more fiber-rich particles by a fouling effect of the mill (Pelgrom et al., 2014), while kernel fragments rich in proteins bodies are concentrated. In addition, it is worth mentioning that rapeseed hulls do not contain any extractable sinapic acid derivatives whereas sunflower hulls contain caffeoylquinic acids in lower but significant amount compared to the kernel.

Regarding particle size distribution of ultrafine RSM and SFM, the effect of milling can be seen in Fig. 2-A1 and B1. In the case of the RSM,

**Table 1**
MS data of phenolic compounds detected in RSM-0.1 methanolic extract.

| Compound | λ max | $[M-H]^- m/z$ | $[M+H]^+ m/z$ | Name |
|---|---|---|---|---|
| 1 | 328 | 294 | 310 | Sinapine |
| 2 | 328 | 771 | n.d. | Kaempferol-3-*O*-sophoroside-7-*O*-*β*-glucopyranoside |
| 3 | 332 | 385 | 207 | Sinapoyl glucopyranoside |
| 4 | 333 | 977 | 979 | Sinapoyl-Kaempferol derivative 1 |
| 5 | 327 | 520 | n.d. | n.d. |
| 6 | 323 | 547 | 665 | n.d. |
| 7 | 307 | 494 | 496 | Cyclic spermidine |
| 8 | 328 | 237 | 207 | Sinapoyl-Kaempferol derivative 2 |
| 9 | 323 | 223 | 207 | Trans-sinapic acid |
| 10 | 323 | 223 | 207 | Cis-sinapic acid |
| 11 | 332 | 977 | 979 | Kaempferol-di-hexoside-sinapoyl-hexoside |
| 12 | 330 | 753 | 777 | Disinapoyl gentiobioside isomer 1 |
| 13 | 328 | 753 | 777 | Disinapoyl gentiobioside isomer 2 |
| 14 | 327 | 977 | 979 | Sinapoyl-Kaempferol derivative 3 |
| 15 | 330 | 591 | 369 | Disinapoyl glucopyranoside |
| 16 | 327 | n.d. | n.d. | n.d. |
| 17 | 326 | 959 | 737 | Trisinapoyl gentiobioside isomer 1 |
| 18 | 328 | 959 | 737 | Trisinapoyl gentiobioside isomer 2 |
| 19 | 328 | 959 | 737 | Trisinapoyl gentiobioside isomer 3 |

Note: n.d.: not determined.

**Table 2**
Composition of SFM-0.5 methanolic extract.

| Compound | λ max | Name |
|---|---|---|
| 1 | 327 | 3-*O*-caffeoylquinic acid |
| 2 | 326 | 4-*O*- and 5-*O*-caffeoylquinic acid |
| 3 | 323 | Caffeic acid |
| 4 | 327 | n.d. |
| 5 | 327 | n.d. |
| 6 | 327 | 5-*O*-*p*-coumaroylquinic acid |
| 7 | 329 | 5-*O*-feruloylquinic acid |
| 8 | 326 | 3,4-di-*O*-caffeoylquinic acid |
| 9 | 328 | 3,5-di-*O*-caffeoylquinic acid |
| 10 | 327 | n.d. |
| 11 | 328 | 4,5-di-*O*-caffeoylquinic acid |

it can be observed that decreasing the grid size has led to the appearance of two populations with modes around 63 µm and 11 µm in case of RSM-0.1. Given the differences in the structure and mechanical properties of kernel and hulls, it was assumed that the first population with a mode of 11 µm was concentrated in small protein bodies disentangled from the cellular matrix of the meal while the second population (mode of 63 µm) was mostly composed of coarse particles of hulls, rich in lignin. Conversely, the presence of three populations (modes comprised between 5 and 350 µm) was only observed after coarse milling of SFM with the 0.5 mm and 0.3 mm grids. As for rapeseed, it was assumed that the two first populations (modes around 5–10 µm and 25–35 µm) were rich in the different protein bodies present in SFM while the last population was composed of coarse fragments of kernel and hulls. When a more intense milling was applied with the 0.2 mm grid, this led to the breakdown of the fiber-rich particles that agglomerate with the protein bodies. Indeed, particles have to be small enough to be detached from the cellular matrix (Hemery et al., 2009), but not too much otherwise agglomeration phenomena may occur and lead to separation issues. Furthermore, intense milling may lead to the breakage of fibrous particles and the contamination of the protein-rich fraction (Schutyser et al., 2015; Wang et al., 2016).

### 3.3. Electrostatic sorting of ultrafine rapeseed and sunflower meals

#### 3.3.1. Influence of the particle size distribution on separation efficiency and composition of fractions

Based on the differences in electrical conductivity and charges, electrostatic sorting (ES) can be used to separate particles that are not initially charged. Here, ultrafine RSM and SFM previously obtained were subjected to a single step electrostatic sorting. After each separation step, four different fractions were recovered: PFe, NFe, PFc and NFc. Globally, $D_{50}$ of PFe fractions were lower than that of NFe fractions, regardless meal type (Fig. 3-A1 and B1). For RSM, $D_{50}$ of PFe and NFe fractions decreased with the grid size (Fig. 3-A1). This trend was only observed with NFe fraction of SFM, as $D_{50}$ of PFe fractions reached a maximum for SFM-0.3. Here, the small particles of hulls might agglomerate with the smallest protein bodies leading to an increase in the $D_{50}$. This was not the case for PFe fraction of SFM-0.2 because most of the finest particles of hulls were lost during the milling step. Regarding the collected fractions PFc and NFc, their higher $D_{50}$ (data not shown) compared to that of the corresponding electrode fractions (PFe and NFe respectively) clearly indicated that the separation was not as efficient as on the electrodes.

**Table 3**
Size, yield and composition of the ultrafine RSM and SFM obtained by milling with different grid sizes.

| Grid size (mm) | RSM | | | SFM | | |
|---|---|---|---|---|---|---|
| | 0.5 | 0.3 | 0.1 | 0.5 | 0.3 | 0.2 |
| Recovery yield (w%) | 96.0 ± 0.6$^a$ | 95.8 ± 0.8$^a$ | 95.9 ± 0.3$^a$ | 97.0 ± 0.2$^a$ | 96.3 ± 0.8$^a$ | 88.8 ± 0.9$^a$ |
| Protein (g/100 g DDM) | 37.9 ± 0.2$^b$ | 39.0 ± 0.1$^c$ | 39.5 ± 0.3$^c$ | 31.3 ± 0.0$^b$ | 30.3 ± 0.1$^c$ | 35.0 ± 0.0$^d$ |
| Sinapine (mg/g DDM) | 11.8 ± 0.3$^d$ | 11.8 ± 0.4$^d$ | 12.4 ± 0.2$^d$ | – | – | – |
| Chlorogenic acid (mg/g DDM) | – | – | – | 17.3 ± 0.3$^e$ | 18.3 ± 0.2$^e$ | 17.3 ± 1.0$^e$ |
| TPC* | 15.6 ± 0.6$^e$ | 15.6 ± 0.3$^e$ | 16.6 ± 0.3$^e$ | 25.3 ± 0.4$^f$ | 24.6 ± 0.2$^f$ | 23.7 ± 1.6$^f$ |
| $D_{50}$ (µm) | 110.9 ± 9.1$^f$ | 69.3 ± 0.6$^g$ | 23.7 ± 1.0$^h$ | 105.5 ± 8.3$^g$ | 65.2 ± 1.1$^h$ | 18.8 ± 0.6$^i$ |

Note: * TPC content was expressed in mg SAE/g DDM and mg CAE/g DDM for RSM and SFM respectively. For each raw material, values followed by same superscript letters are not significantly different, $p \leq 0.05$. Values are mean ± SD (n = 3).

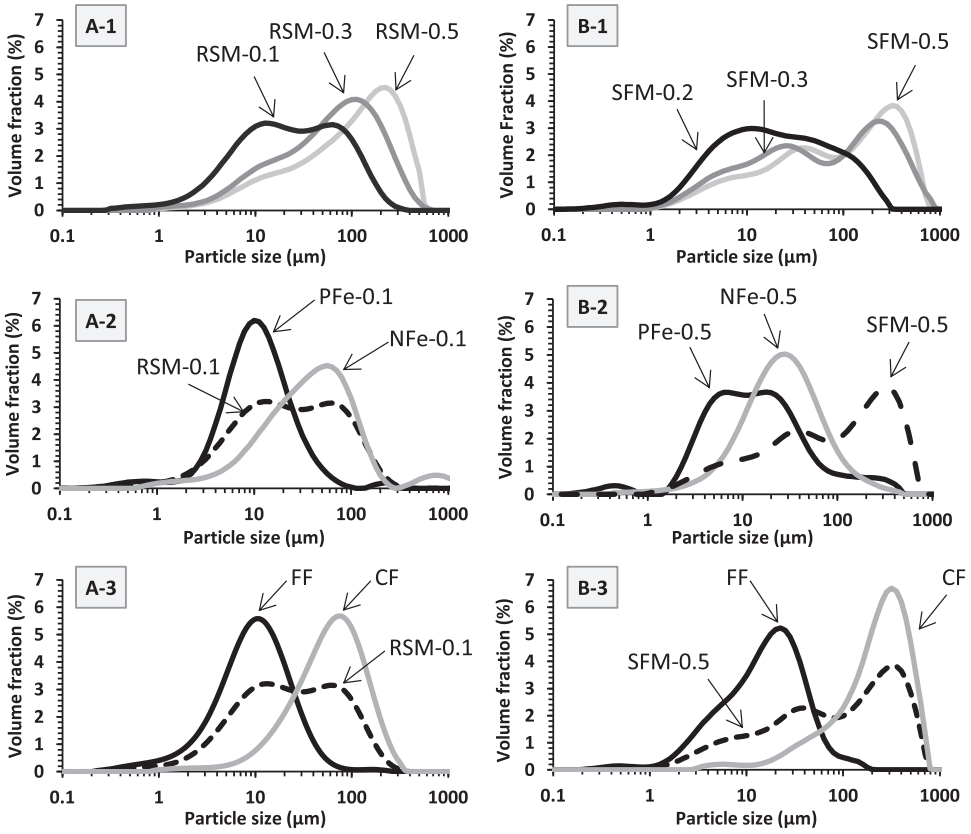


**Fig. 2.** Particle size distributions of ultrafine RSM (A) and SFM (B) samples, obtained by milling with different grid sizes (1), then after electrostatic sorting-ES (2) and turbo separation-TS (3).

Visually, positive fractions PFe and PFc of both meals were "whiter" than the corresponding negative ones (brownish), thus suggesting a partial separation of protein-rich kernel particles from the others. This was confirmed by the highest protein and TPC content of PFe fractions and, conversely, the lowest in the negative fractions NFe (Fig. 3-A2 and B2). Indeed, as already pointed out by others, when the particles pass through the charging line, the ones containing more proteins are positively charged and agglomerate onto the negatively charged electrode (Hemery et al., 2011; Wang et al., 2016). Moreover, these results clearly showed that the phenolic compounds were separated with the proteins. This means that in RSM and SFM, most of the phenolic compounds are intimately linked to the proteins and this complex cannot be broken by milling and ES. With regard to the protein and TPC content of collected fractions PFc and NFc, they turned out to be not very different from that of the initial fractions F0 (Fig. 3-A2 and B2). In fact, when the electrodes are covered, the attraction of the other particles towards the electrodes becomes more and more difficult, due to the shielding, and the separation ends up being ineffective with a nearly statistical distribution of the particles into the collecting jars. Finally, it was noticed that there was no qualitative but quantitative difference in the individual phenolic compounds (sinapine and 5-CQA were selected as specific markers of RSM and SFM respectively) of each fraction that confirms the higher concentration of phenolics in the kernels than in the hulls (Baumert et al., 2005; Fang et al., 2012; Liu et al., 2012; Weisz et al., 2009).

During this study, it was observed that there is an influence of the particle size distribution on ES. Indeed, for the RSM, the highest increase of 53% in protein (59.1 ± 0.4 g/100 g DDM) and 57% in TPC (25.8 ± 0.1 mg SAE/g DDM) content was observed in the PFe fraction obtained from the finest fraction (RSM-0.1; $D_{50}$ = 23.7 ± 1.0 µm). This fraction was named "RSM-PFe-0.1". In the RSM-0.1, the protein bodies linked to the phenolic compounds are disentangled from the cellular matrix and are more easily charged and recovered. This separation can be clearly seen in Fig. 2-A2. For the SFM, the highest increase was seen in the PFe fraction of SFM-0.2. However, due to the loss of most of the hulls during the milling step, this fraction was not taken into account. Finally, the highest increase of 96% in protein (61.1 ± 0.0 g/100 g DDM) and 80% in TPC (45.8 ± 1.1 mg CAE/g DDM) content was obtained in the PFe fraction achieved from the more coarsely meal (SFM-0.5; $D_{50}$ = 105 ± 8.3 µm). This fraction was named "SFM-PFe-0.5". For this meal, a coarse milling allows the disentanglement of the complex protein-phenolic compounds from the other constituents. On the other hand, a more intense milling led to agglomeration phenomena and to a less efficient separation. The separation of the different populations present in the SFM-0.5 can be seen in Fig. 2-B2, while two distinct populations are observed in SFM-PFe-0.5. This might suggest the presence of different protein bodies on this meal. The low $D_{50}$ of the NFe fraction ($D_{50}$ = 22 ± 0.8 µm) is representative to the small fiber-rich constituents of the hulls that were more easily broken down and recovered in this fraction, explaining the low protein (14.7 ± 0.0 g/100 g DDM) and TPC (7.9 ± 0.2 mg CAE/g DDM) contents. Finally, the "lignin" content of the PFe-0.1, PFe-0.5 and their NFe fractions was determined and compared against the initial F0 fractions (Table 4). As expected, PFe fractions did not contain lignin and, conversely, their respective NFe fractions were highly concentrated in lignin (31.4 ± 0.5 g/100 g DDM and 26.2 ± 0.8 g/100 g DDM for the RSM and SFM respectively). These results confirm the efficient separation of the protein bodies with the phenolic compounds from the other constituents of the meals after ES.

### 3.3.2. *Recovery yield and recycling steps*

Even though the highest protein and TPC contents were found in RSM-PFe-0.1 and SFM-PFe-0.5 fractions, their recovery yield remained however low, around 9% and 18% respectively. To enhance the

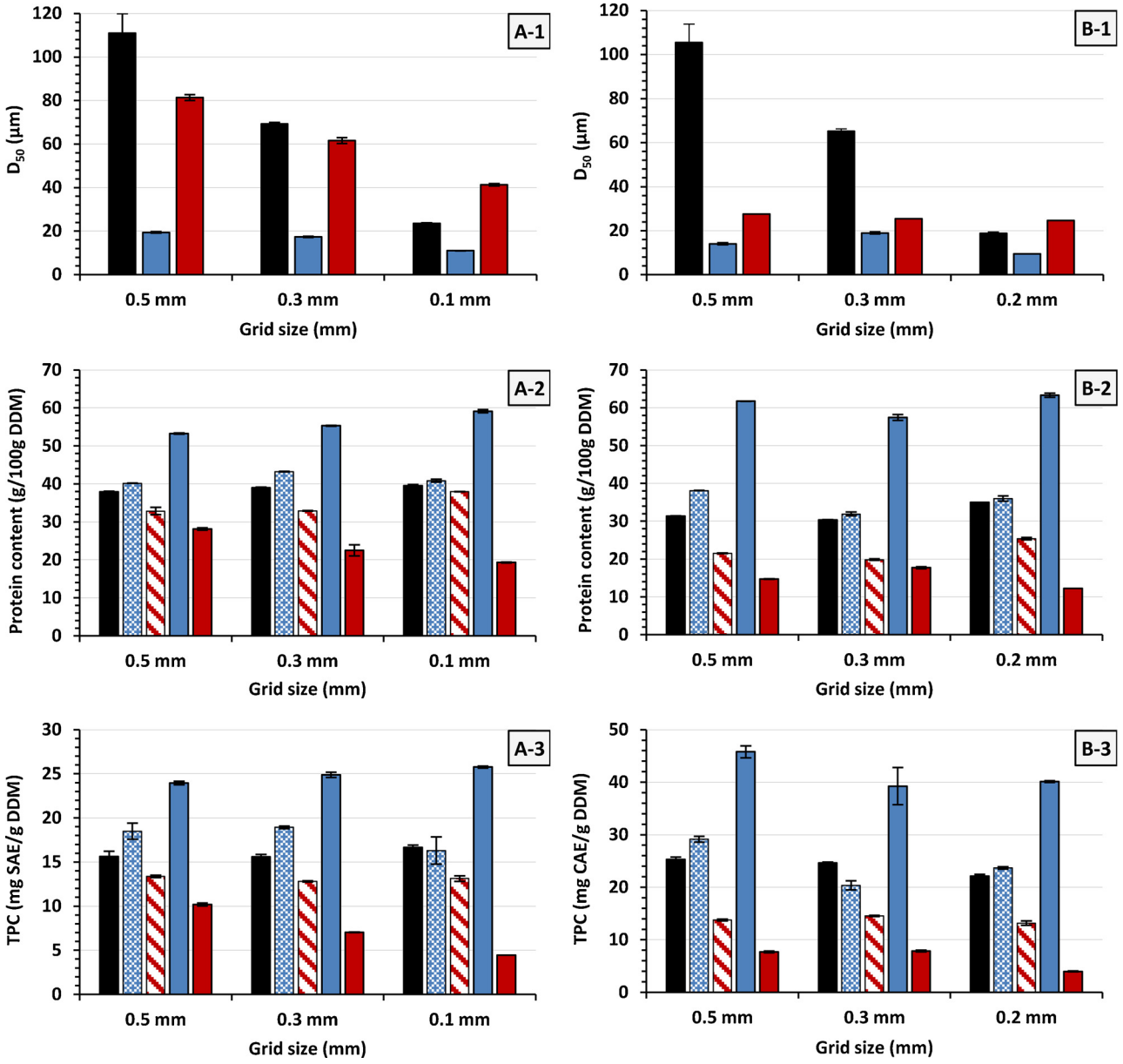


**Fig. 3.** Characterization of RSM (A) and SFM (B) fractions after a single-step electrostatic sorting-ES, as function of the starting meal ground with different grid sizes: $D_{50}$ (1), protein (2) and TPC (3) content. ■ F0, ▩ PFc, ▨ NFc, ■ PFe and ■ NFe. For each material, values followed by same superscript letters are not significantly different, $p \leq 0.05$. Values are means ± SD (n = 3).

**Table 4**
Physicochemical characterization of PFe-0.1, PFe-0.5 and their respective NFe fractions obtained from electrostatic sorting-ES of RSM and SFM.

| Physico-chemical characteristics | RSM | | | SFM | | |
|---|---|---|---|---|---|---|
| | F0-0.1 | PFe-0.1 | NFe-0.1 | F0-0.5 | PFe-0.5 | NFe-0.5 |
| $D_{50}$ (µm) | 23.7 ± 1.0$^{a}$ | 11.0 ± 0.0$^{b}$ | 41.3 ± 0.6$^{c}$ | 105.5 ± 8.3$^{a}$ | 14.1 ± 0.6$^{b}$ | 27.6 ± 0.0$^{c}$ |
| Recovery yield (g/100 g) | – | 9.2 ± 1.0$^{d}$ | 4.8 ± 2.1$^{e}$ | – | 18.0** | 2.7** |
| Protein content (g/100 g DDM) | 36.7 ± 0.3$^{f}$ | 59.1 ± 0.4$^{g}$ | 19.3 ± 0.1$^{h}$ | 31.3 ± 0.0$^{d}$ | 61.7 ± 0.0$^{e}$ | 14.7 ± 0.1$^{f}$ |
| Sinapine (mg/g DDM) | 11.6 ± 0.2$^{i}$ | 18.8 ± 0.1$^{j}$ | 3.0 ± 0.1$^{k}$ | – | – | – |
| Chlorogenic acid (mg/g DDM) | – | – | – | 17.3 ± 0.3$^{g}$ | 32.7 ± 0.8$^{h}$ | 5.5 ± 0.1$^{i}$ |
| TPC content* | 15.5 ± 0.3$^{l}$ | 25.8 ± 0.1$^{m}$ | 4.2 ± 0.0$^{n}$ | 25.3 ± 0.4$^{j}$ | 45.8 ± 1.1$^{k}$ | 7.7 ± 0.2$^{l}$ |
| Lignin (g/100 g DDM) | 10.9 ± 0.4$^{o}$ | 0.0 ± 0.0$^{p}$ | 31.4 ± 0.5$^{q}$ | 15.2 ± 0.2$^{m}$ | 0.0 ± 0.0$^{n}$ | 26.2 ± 0.8$^{o}$ |

Note: *TPC content was expressed in mg SAE/g DDM and mg CAE/g DDM for RSM and SFM respectively. For each raw material, values followed by same superscript letters are not significantly different, $p \leq 0.05$. Values are means ± SD (n = 3), except for ** (single experiment).

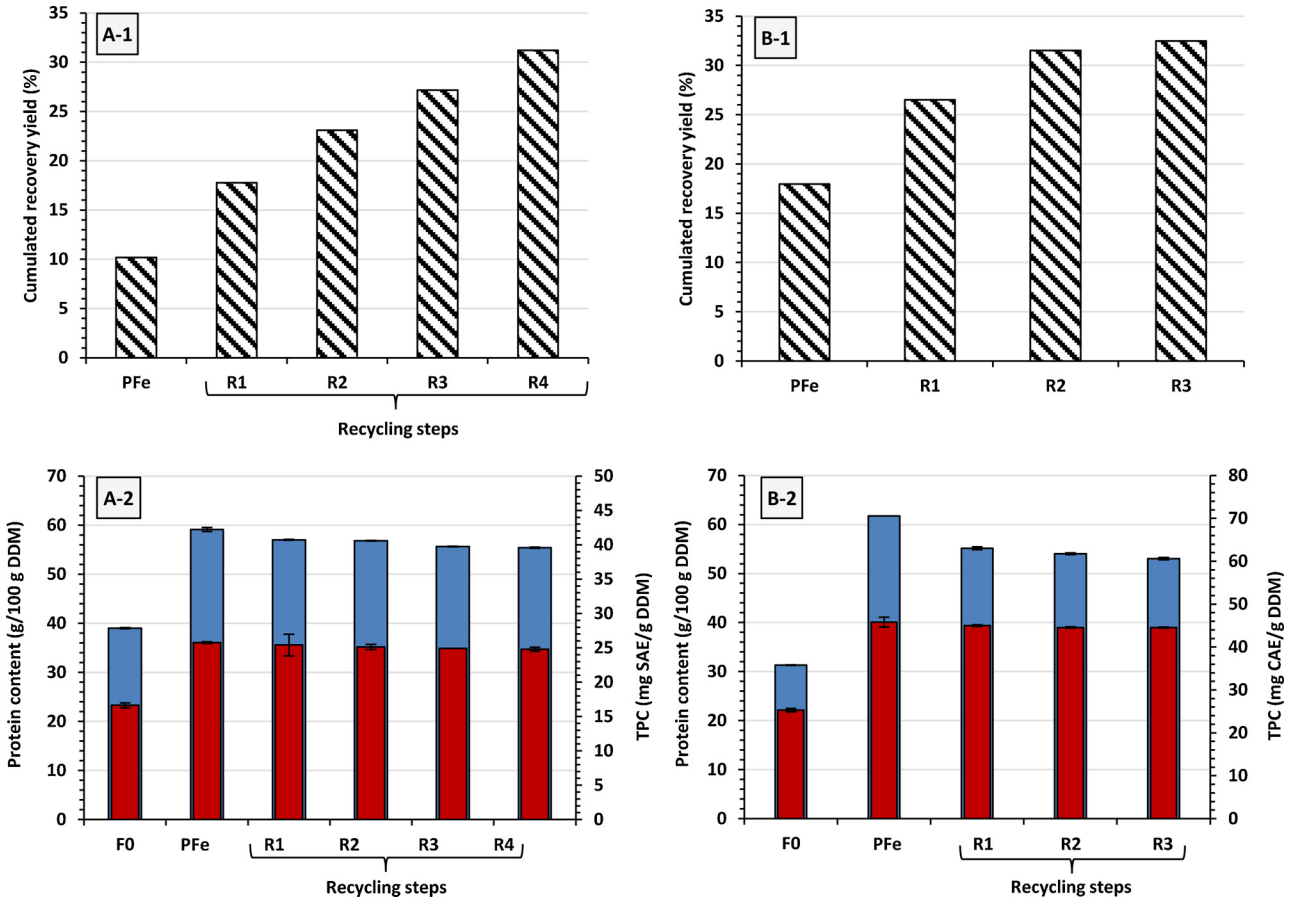


**Fig. 4.** Cumulated recovery yield (■) of RSM-0.1-PFe (A-1) and SFM-0.5-PFe (B-1). Evolution of Protein (■) and TPC (■) content of RSM-0.1-PFe (A-1) and SFM-0.5-PFe (B-1) as function of recycling steps. For each material, values followed by same superscript letters are not significantly different, $p \leq 0.05$. Values are means ± SD (n = 3).

recovery yield of these fractions, multiple recycling steps of the collected fractions PFc and NFc were done, following the methodology applied to lupine by Wang and coworkers (Wang et al., 2016). The results are summarized in Fig. 4. The cumulated recovery yield increased from 9% to 31% after four cycles for RSM-PFe-0.1(Fig. 4-A1) and from 18% to 32% after three cycles for SFM-PFe-0.5 (Fig. 4-B1). However, this increase was accompanied by a steady decline of the protein content (overall 6% and 14% respectively), while at the same time, TPC remained stable (Fig. 4-A2 and B2). This trend was also observed by (Wang et al., 2016). Indeed, the composition of the different particles is not the same in the meal, and the finest particles that contain more proteins and that are more easily charged are first recovered in the initial separation step. Then, a progressive exhaustion of protein-rich and negatively charged particles occurs in the course of recycling, until the yield of PFe fraction becomes insignificant (data not shown). Although electrostatic sorting of rapeseed and sunflower meals has been already studied by Barakat et al., 2015 and Basset et al., 2016 with the same apparatus operating in identical conditions, the authors were not interested in the fractions recovered on the electrodes but only in the fractions recovered in the collecting jars under the electrodes (what we called here NFc and PFc). Moreover, they followed a "cascade" approach that could be summarized as follows: from the initial fraction F0, two fractions, the positively charged particles F1A− and the negatively charged particles F1B+, were recovered in separate collecting jars after the first sorting. These two fractions underwent separately a second separation step, leading to four different fractions: F2A− and F2A+ fractions from F1A−, and F2B+ and F2B− fractions from F1B+. A third separation step was carried out on fractions F2A- and F2B+, yielding on one side F3A− and F3A+ fractions, and on the other side F3B− and F3B+ fractions respectively. Applied to rapeseed meal (Basset et al., 2016), the electrostatic sorting through this "cascade" approach led to an increase in the protein content from 37% in the F0 fraction ($D_{50}$ = 89.7 µm), to 51% in the F3B+ fraction ($D_{50}$ = 89.3 µm, yield = 43%)

Thus, compared to the above, the present methodology focusing on the positive electrode fraction and on the recycling of unseparated particles allows the recovery of fractions of much higher content in proteins and phenolics.

### 3.4. Turbo separation of the ultrafine rapeseed and sunflower meal

#### 3.4.1. Influence of ultrafine milling and separation wheel speed

The TS technology allows particles separation according to their size and density. This approach has been successfully applied to produce protein concentrates from lupine, field peas, pea beans, northern beans, faba beans, lima beans, mung beans and lentils (Sosulski and Youngs, 1979). It was observed that the small protein bodies were mostly recovered in the finest fractions while others fragments rich in lignin and/or fibers were recovered in the coarsest fractions. Here, ultra-fine RSM and SFM were subjected to turbo separation. Three different rotation speed of the classifier wheel (3000 rpm, 5000 rpm and 7000 rpm) were tested. After each separation step, two different fractions were recovered: a fine fraction named FF and a coarse fraction named CF. Whatever the rotation speed, FF were "whiter" than CF. Again, it was expected that some of the particles from the kernels (that are "whiter") could be separated from hull particles by TS. The protein and TPC contents of the different fractions as a function of classifier wheel speed can be seen in Fig. 5. Overall, FF have higher protein content compared

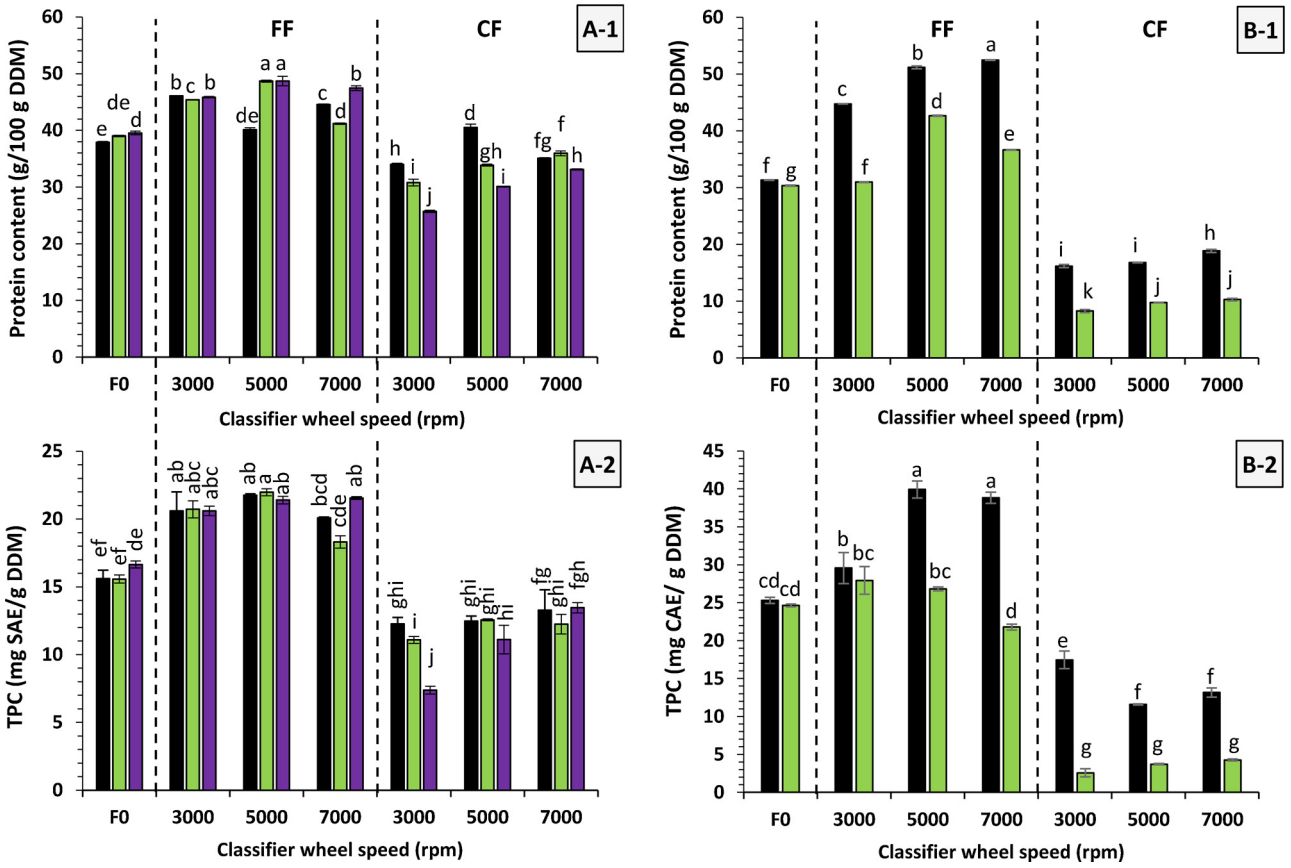


**Fig. 5.** Protein and TPC content of RSM (A-1 and A-2) and SFM (B-1 and B-2) fine fractions (FF) and coarse fractions (CF) after turbo-separation-TS. Grid size (■) 0.5 mm, (■) 0.3 mm, (■) 0.1 mm. (For each material, values followed by same superscript letters are not significantly different, $p \leq 0.05$).Values are means ± SD (n = 3).

to CF, whatever the meal and the rotation speed. Indeed, the lighter and small protein bodies are recovered in FF. Again, fractions containing more protein also contained more TPC, meaning that protein-phenolic complexes cannot be separated; neither by UFM nor by TS. As for electrostatic separation, there was no qualitative but quantitative difference in the individual phenolic compounds of each fraction (data not shown).

Regarding RSM, although the influence of the rotation speed on the separation was different for each ultra-fine meal, the TPC and protein content of CF, globally increase with classifier wheel speed (Fig. 5-A1). This is likely due to the contamination of CF by big kernel particles. Increasing classifier wheel speed also led to lower recovery yields of FF

**Table 5**
Particle size distribution, yield and composition of the fine fraction (FF) of RSM and SFM obtained by turbo-separation-TS using different classifier wheel speeds.

| Grid size (mm) | | Wheel speed (rpm) | $D_{50}$ (μm) | Protein (g/100 g DDM) | TPC* | FF yield (w%) |
|---|---|---|---|---|---|---|
| SFM | 0.5 | F0 | 105.5 ± 8.3 | 31.3 ± 0.1 | 25.3 ± 0.4 | – |
| | | 3000 | 30.4 ± 0.7 | 44.7 ± 0.0 | 29.6 ± 2.1 | 47.0** |
| | | 5000 | 20.4 ± 0.3 | 51.2 ± 0.3 | 39.9 ± 1.1 | 30.0** |
| | | 7000 | 17.5 ± 0.2 | 52.4 ± 0.1 | 38.8 ± 0.8 | 22.0** |
| | 0.3 | F0 | 65.2 ± 1.1 | 30.3 ± 0.1 | 24.6 ± 0.2 | – |
| | | 3000 | 53.2 ± 1.7 | 31.0 ± 0.1 | 27.9 ± 1.8 | 70.0** |
| | | 5000 | 35.6 ± 6.0 | 42.6 ± 0.1 | 26.8 ± 0.3 | 72.0** |
| | | 7000 | 47.8 ± 0.0 | 36.6 ± 0.0 | 21.8 ± 0.4 | 82.0** |
| RSM | 0.5 | F0 | 110.9 ± 9.1 | 37.9 ± 0.2 | 15.6 ± 0.6 | – |
| | | 3000 | 22.2 ± 0.4 | 46.1 ± 0.0 | 20.6 ± 1.4 | 34.1 ± 3.2 |
| | | 5000 | 23.5 ± 0.6 | 40.1 ± 0.4 | 21.8 ± 0.1 | 20.2 ± 3.7 |
| | | 7000 | 11.6 ± 0.2 | 44.6 ± 0.1 | 20.1 ± 0.1 | 4.0 ± 1.7 |
| | 0.3 | F0 | 69.3 ± 0.6 | 39.0 ± 0.1 | 15.6 ± 0.3 | – |
| | | 3000 | 22.6 ± 0.8 | 45.4 ± 0.1 | 20.7 ± 0.4 | 37.9 ± 3.8 |
| | | 5000 | 12.0 ± 0.5 | 48.7 ± 0.2 | 22.0 ± 0.2 | 23.5 ± 0.7 |
| | | 7000 | 11.8 ± 0.6 | 41.2 ± 0.1 | 18.3 ± 0.4 | 5.4 ± 0.2 |
| | 0.1 | F0 | 23.7 ± 1.0 | 39.5 ± 0.3 | 16.6 ± 0.2 | – |
| | | 3000 | 16.9 ± 0.1 | 45.9 ± 0.1 | 20.6 ± 0.2 | 58.9 ± 0.8 |
| | | 5000 | 10.3 ± 0.1 | 48.7 ± 0.8 | 21.4 ± 0.3 | 40.7 ± 0.6 |
| | | 7000 | 8.6 ± 0.1 | 47.4 ± 0.4 | 21.6 ± 0.1 | 25.1 ± 1.5 |

**Note:** *TPC content was expressed in mg SAE/g DDM and mg CAE/g DDM for RSM and SFM respectively. Values are mean ± SD (n = 3), except for ** (single experiment)

**Table 6**
Chemical characterization of the fine fraction (FF) of RSM-0.1 and SFM-0.5 obtained at 5000 rpm and their respective coarse fraction (CF).

| Physico-chemical characteristics | RSM | | | SFM | | |
|---|---|---|---|---|---|---|
| | F0-0.1 | FF-0.1–5000 | CF-0.1–5000 | F0-0.5 | FF-0.5–5000 | CF-0.5–5000 |
| $D_{50}$ (µm) | 23.7 ± 1.0$^{a}$ | 10.3 ± 0.1$^{b}$ | 64.9 ± 0.2$^{c}$ | 105.5 ± 8.3$^{a}$ | 20.4 ± 0.3$^{b}$ | 248.9 ± 3.3$^{c}$ |
| Recovery yield (g/100 g) | – | 40.7 ± 0.6$^{d}$ | 42.4 ± 0.6$^{e}$ | – | 30.0** | 59.0** |
| Protein content (g/100 g DDM) | 36.7 ± 0.3$^{f}$ | 48.7 ± 0.8$^{g}$ | 30.1 ± 0.4$^{h}$ | 31.3 ± 0.0$^{d}$ | 51.2 ± 0.3$^{e}$ | 16.8 ± 0.0$^{f}$ |
| Sinapine (mg/g DDM) | 11.6 ± 0.2$^{i}$ | 16.2 ± 0.1$^{j}$ | 8.2 ± 0.9$^{k}$ | – | – | – |
| Chlorogenic acid (mg/g DDM) | – | – | – | 17.3 ± 0.3$^{g}$ | 29.3 ± 0.7$^{h}$ | 8.9 ± 0.0$^{i}$ |
| TPC* | 15.5 ± 0.3$^{l}$ | 21.4 ± 0.3$^{m}$ | 11.1 ± 1.0$^{n}$ | 25.3 ± 0.4$^{j}$ | 39.9 ± 1.1$^{k}$ | 11.6 ± 0.1$^{l}$ |
| Lignin (g/100 g DDM) | 10.9 ± 0.4$^{\circ}$ | 0.0 ± 0.0$^{p}$ | 30.5 ± 0.9$^{q}$ | 15.2 ± 0.2$^{m}$ | 0.0 ± 0.0$^{n}$ | 25.2 ± 0.8$^{\circ}$ |

Note: *TPC content was expressed in mg SAE/g DDM and mg CAE/g DDM for RSM and SFM respectively. For each raw material, values followed by same superscript letters are not significantly different, $p \leq 0.05$. Values are means ± SD (n = 3), except for ** (single experiment).

(Table 5). Compared to initial F0 fractions, the highest protein and TPC contents were obtained at 5000 rpm with the ultra-fine RSM-0.3 and RSM-0.1 (Fig. 5-A1 and 5-A2, Table 5). However, the recovery yield of the FF of the RSM-0.3 was lower (23.5 ± 0.7%) than that of RSM-0.1 (40.7 ± 0.6%). At the particle size distribution of the RSM-0.1, most of the protein bodies were disentangles from the cellular matrix, making easier their recovery. Finally, the separation of the two different populations of RSM-0.1 can be clearly seen in Fig. 2-A3.

The influence of the classifier wheel rotation speed was also observed for ultra-fine SFM. For the one obtained with the 0.5 mm grid, higher rotation speeds led to an increase in the TPC (from 29.6 ± 2.1 mg CAE/g DDM to 38.8 ± 0.8 mg CAE/g DDM) and the protein content (from 44.7 ± 0.0 g/100 g DDM to 52.4 ± 0.1 g/100 g DDM). Nevertheless, this increase was followed by a decline in the recovery yield from 47.0% to 22.0%. As for the fine fractions obtained from ultra-fine SFM-0.3, the increase in protein and the TPC contents were lower, owing to the contamination of protein bodies by small fiber-rich hull particles. Moreover, the TPC and protein contents also

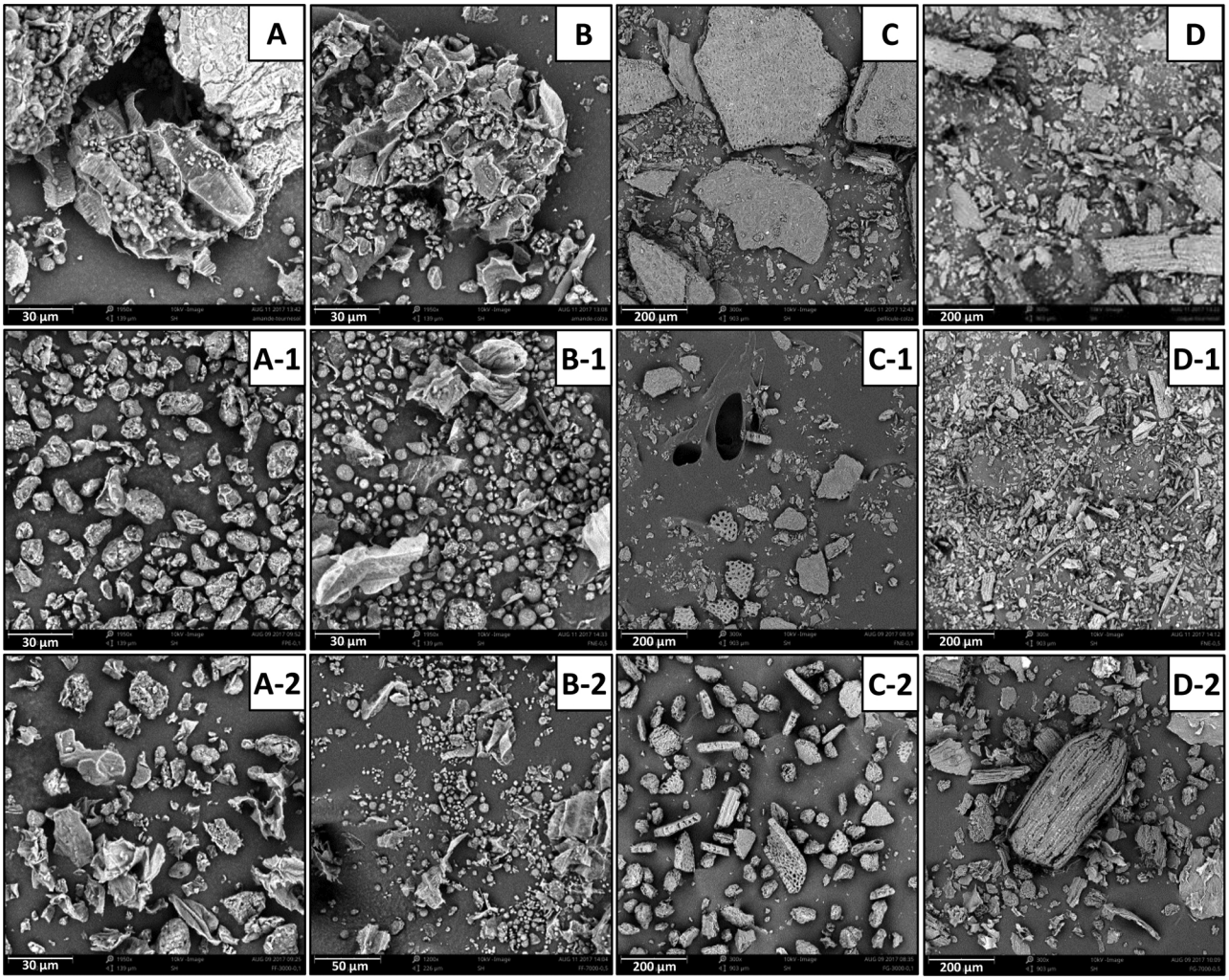


**Fig. 6.** SEM images of raw and processed samples: Delipidated and milled rapeseed kernels (A), sunflower kernels (B), rapeseed hulls (C) and sunflower hulls (D); Fractions obtained after electrostatic sorting-ES: RSM-PFe-0.1 (A-1), SFM-PFe-0.5 (B-1), RSM-NFe-0.1 (C-1) and SFM-FNe-0.5 (D-1); Fractions obtained after turbo-separation-TS: RSM-FF-0.5 (A-2), SFM-FF-0.5 (B-2), RSM-CF-0.5 (C-2) and SFM-CF-0.5 (D-2).

decreased for rotation speed higher than 5000 rpm. The smaller particles of this ultra-fine meal have been likely more exposed to the air with, as a consequence, the accelerated oxidation of phenolic compounds. When SFM-0.2 was submitted to turbo-separation, all the meal was recovered in the fine fraction. It means that grinding sunflower meal with a 0.2 mm grid led to particles so small that TS becomes inefficient. The best compromise between purity (protein content) and recovery yield was achieved at 5000 rpm for SFM-0.5. In the corresponding fine fraction, it was noticed an increase of 67.4% in proteins (51.2 ± 0.3 g/100 g DDM), 53.3% (39.9 ± 1.1 mg CAE/g DDM) in TPC, and a recovery yield of 30.0%. Again, the separation of the two different populations of SFM-0.5 can be clearly seen in Fig. 2-B3.

The lignin content of fine and coarse fractions obtained at 5000 rpm from RSM-0.1 and SFM-0.5 were also determined (Table 6). As expected, only the coarse fractions contained lignin in significant amounts, whereas it was totally lacking in the fine fractions. To conclude, the results presented above clearly demonstrated that turbo-separation applied in appropriate conditions to RSM and SFM displaying adequate particle size distribution, allows the production of a fine fraction concentrated in protein bodies (along with phenolics), and a coarse fraction mostly constituted of hulls. A phenomenon particularly marked in the case of SFM.

### 3.5. SEM analysis of the different fractions

The separation of the different constituents of the meals by ES and TS and the different fractions recovered can be also seen in Fig. 6. The SEM images of delipidated and milled kernels (Fig. 6A and 6B) showed the small globular protein bodies (3–6 µm) stored inside cellular matrices. SEM images of delipidated and milled rapeseed hulls (Fig. 6C) show their reticulated morphology and the sunflower hulls (Fig. 6D) are present as more fiber-rich constituents. After ultrafine milling and electrostatic sorting, the globular protein bodies are concentrated in the positive fraction RSM-PFe-0.1 (Fig. 6-A1) and SFM-PFe-0.5 (Fig. 6-B1), whereas hull particles are recovered in the negative fractions NFe-0.1 (Fig. 6-C1) and NFe-0.5 (Fig. 6-D1). Finally, regarding turbo-separation, fine fractions of both meals are enriched in protein bodies (Fig. 6-A2 and 6-B2) and, conversely, coarse fractions mostly contain hulls (Fig. 6-C2 and D2).

## 4. Conclusions

A properly milling combined with ES or TS technologies allows the recovery of fractions with high protein and phenolic contents from RSM and SFM. However, even if ES leads to the recovery of fractions with higher purities, recycling steps remain essential to increase the overall recovery yield up to 30%. This recycling has, however, the drawback of slightly lowering the protein content and of increasing the energy consumption of the process. To avoid recycling, some modifications of the apparatus could be considered such as the enlargement of the electrodes or the continuous and automatic recovery of particles onto the electrodes. Regardless the type of meal, phenolic compounds were separated with the proteins after ES or TS. This implies that the proteins are somehow linked to the phenolic compounds and that they cannot be separated by dry fractionation processes. Therefore, if the isolation of pure proteins or phenolics is an objective, dry fractionation should be regarded as a mild pre-purification process prior to solid-liquid extraction steps. On the other hand, as depicted by several authors, the association of proteins and phenolic compounds can be taken as an advantage, since they could act together as emulsifiers displaying strong antioxidant activities. Moreover, in the area of biomaterials, phenolics (especially those bearing several aromatic hydroxyls) might also act as endogenous protein cross-linkers, thus being a green and safe alternative to aldehydes such as formalin, glyoxal or glutaraldehyde. Finally, the extraction of phenolics may represent an interesting solution allowing the simultaneous production of bioactive molecules (e.g. antioxidants, antimicrobials) or building blocks for polymer synthesis in one hand, and phenolic-free protein fractions for the production of isolates or peptides on the other hand.

## Competing interest

The authors have declared no conflict of interest.

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