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*Full length paper*

# **Multiobjective decision making strategy for selective albumin extraction from a rapeseed cold-pressed meal based on Rough Set approach**

**Sophie Beaubier<sup>1</sup>, Claire Defaix<sup>1,2</sup>, Sara Albe-Slabi<sup>1,2</sup>, Arnaud Aymes<sup>1</sup>, Olivier Galet<sup>2</sup>, Frantz Fournier<sup>1</sup>, and Romain Kapel<sup>1\*</sup>**

<sup>1</sup> Université de Lorraine, CNRS, LRGP, F-54000 Nancy, France

<sup>2</sup> Avril SCA, 11 rue de Monceau, F-75008 Paris, France

\* Corresponding author: [romain.kapel@univ-lorraine.fr](mailto:romain.kapel@univ-lorraine.fr), 13 rue du bois de la Champelle 54500 Vandoeuvre-lès-Nancy

[sophie.beaubier@univ-lorraine.fr](mailto:sophie.beaubier@univ-lorraine.fr)

[claire.defaix03@orange.fr](mailto:claire.defaix03@orange.fr)

[sara.albe-slabi@univ-lorraine.fr](mailto:sara.albe-slabi@univ-lorraine.fr)

[arnaud.aymes@univ-lorraine.fr](mailto:arnaud.aymes@univ-lorraine.fr)

[olivier.galet@groupeavril.com](mailto:olivier.galet@groupeavril.com)

[frantz.fournier@univ-lorraine.fr](mailto:frantz.fournier@univ-lorraine.fr)

**Abstract :**

This article proposes the application of multiobjective decision making strategy for selective albumin extraction by Pareto's domain determination and ranking by Decision Engineering tool based on the Rough Set approach. In this study, both competing **process performance indicators** of rapeseed albumin extraction and quality of the solid residue remaining from this process were considered for an **overall** valorization of the rapeseed cold-pressed meal. The impact of extraction pH and NaCl concentration on three **process performance indicators** including the albumin extraction yield, the albumin content in the extract, and the phytic acid content in the remaining solid residue was studied. The results of the optimization approach showed that the high selectivity of rapeseed albumin extraction was to the detriment of the productivity of the process and the quality of the solid residue. The optimal conditions for selective rapeseed albumin extraction while producing a high-quality solid residue were identified at pH 2.0 and 0.11 mol.L<sup>-1</sup> of NaCl concentration. Upon these conditions, good albumin extraction yield was achieved (55.5%) with a high selectivity (97.9% of albumins in the extract). The isolate obtained after purification exhibited suitable color, high solubility, promising emulsifying properties and good foaming properties, which is of interest for food applications. Besides, the identifying extraction conditions yielded a protein-rich solid residue (35.6% on dry matter basis) with reduced phytic acid content (3.6% on dry matter basis), which can be used for feed applications.

**Keywords:** Pareto optimization; Multiobjective decision making; Rough Set approach; Plant protein extraction; Rapeseed albumins; Valorization.

**List of abbreviations:**

ANOVA - analysis of variance

DoE - design of experiments

FAO - Food and Agriculture Organization

MCDM - multicriteria decision-making

pI - isoelectric point

R<sup>2</sup> - coefficient of determination

RA - rapeseed albumins

RSD - relative standard deviation,

SE-HPLC - size exclusion high performance liquid chromatography

TFA - trifluoroacetic acid

## 1. Introduction

Global production of rapeseed increased from 39.6 million tons in 2000 to over 70 million tons in 2019 (FAO, 2019) taking the third place in worldwide production of oilseed. The solid residue remaining after the oil extraction process, called rapeseed meal, contains a high amount of protein (from 30 to 50% on dry matter basis) (Aider & Barbana, 2011; Perera et al. 2016; von der Haar et al. 2014; Yoshie-Stark et al. 2008). It is therefore currently used as a feed supplement rich in protein and energy for livestock.

The two main classes of rapeseed proteins are 12S globulins (cruciferins) and 2S albumins (napins) (von der Haar et al. 2014; Wanasundara, 2011). Rapeseed globulins have a high-molecular weight (230-350 kDa) and an isoelectric point (pI) at pH 7.2, whereas rapeseed albumins are low-molecular weight proteins (12 to 15 kDa) with an average pI around 11 (Akbari & Wu, 2015; Cheung et al. 2014; Perera et al. 2016; Tan et al. 2011). Overall, rapeseed proteins have a well-balanced composition in amino acids (Gueguen et al. 2016) and good functional properties such as foaming and emulsifying (Aider & Barbana, 2011; Yoshie-Stark et al. 2008). For these reasons, rapeseed meal recently gains a great interest as a promising source of plant proteins for human nutrition.

For food applications such as the production of the nutritional, functional, or stabilization ingredients, isolate grade products (> 90% of protein on dry matter basis) are typically used, produced in a two-step process. Many reports describe production process for total protein isolates from rapeseed meal. The common strategy consists of alkali extraction (pH 9.0-12.0) followed by protein purification by acidic precipitation (pH 2.5-4.0) (Aider & Barbana, 2011; Aluko & McIntosh, 2001; Fetzer et al. 2019; Tan et al. 2011). Nevertheless, this strategy presents several drawbacks: (i) a poor purification yield (about 30%) due to an important

loss of albumins that remain soluble in the liquid phase of acidic precipitation (Akbari & Wu, 2015; Blaicher et al. 1983; Chen & Rohani, 1992); (ii) the production of protein isolates characterized by limited functional and nutritional properties due to the irreversible denaturation of proteins during alkaline extraction and again the loss of albumins which are well-soluble and have a high sulfur amino acid content (Pedroche et al. 2004; Tan et al. 2011); and (iii) the by-production of a large amount of poor-quality solid residue remaining after the protein extraction step. This remaining solid is indeed considerably reduced in proteins (about 50% of initial content), and enriched in antinutritional compounds, particularly in phytic acid (Hudson, 1994; Tan et al. 2011). Therefore, an alternative strategy that considers protein quality, recovery and the remaining residue value must be addressed.

Among the two main protein fractions, rapeseed albumins (RA) present a better amino acid composition (according to the recommendations of FAO/WHO/UNU 2007; World Health Organization, & United Nations University, 2007) and are rich in sulfur compounds which is noteworthy for plant proteins (Gueguen et al. 2016; Youle and Huang, 1981). Besides, some studies reported their promising functional properties (Aider & Barbana, 2011; Aluko & McIntosh, 2001; Krause & Schwenke, 2001; Yoshie-Stark et al. 2008) and biological activities, such as antifungal or antimicrobial activities (Nioi et al. 2012). In rapeseed meal, RA represent from 25 to 40% of the total proteins (Wanasundara, 2011), the others being mainly the rapeseed globulins, which are less interesting for food applications in comparison.

Interestingly, some authors reported selective extraction of RA upon acidic conditions (Nioi et al. 2012; Perera et al. 2016; Wanasundara et al. 2012; Wanasundara & McIntosh, 2013). This strategy of acidic extraction also allowed the production of solid residue with a high amount of nitrogen (mainly globulins). Besides, the phytic acid can be removed efficiently at acidic

pH extraction (Akbari & Wu, 2015; Blaicher et al. 1983; Serraino & Thompson, 1984; Zhou et al. 1990). Hence, it will result in a high-quality residue with interest for feed application.

The selective extraction can be impacted by various conditions such as the pH, the temperature, the meal/liquid ratio, and the ionic strength (NaCl concentration) (Du et al. 2018; Fetzer et al. 2018; Oomah et al. 1994). **The four main performance indicators of the selective extraction process are the albumin extraction yield, the albumin content in the extract, and the phytic acid and the protein content in the solid residue.** To date, plant protein extraction optimization mainly consists in identifying the extraction conditions that maximize the extraction yield and/or the protein content. To do so, design of experiments (DoE) is classically applied (Du et al. 2018; Jarpa-Parra et al. 2014; Mizubuti et al. 2000). However, to our knowledge, no optimization study has been reported considering simultaneously **process performance indicators** of RA extraction and quality of the solid residue. **This can be explained by conflicting extraction performance indicators. Indeed, the maximization of protein extraction leads to the by-production of low-value residual meal that is poor in proteins and enriched in antinutritional phytic acid. This considerably limits its re-valorization in animal feeding.**

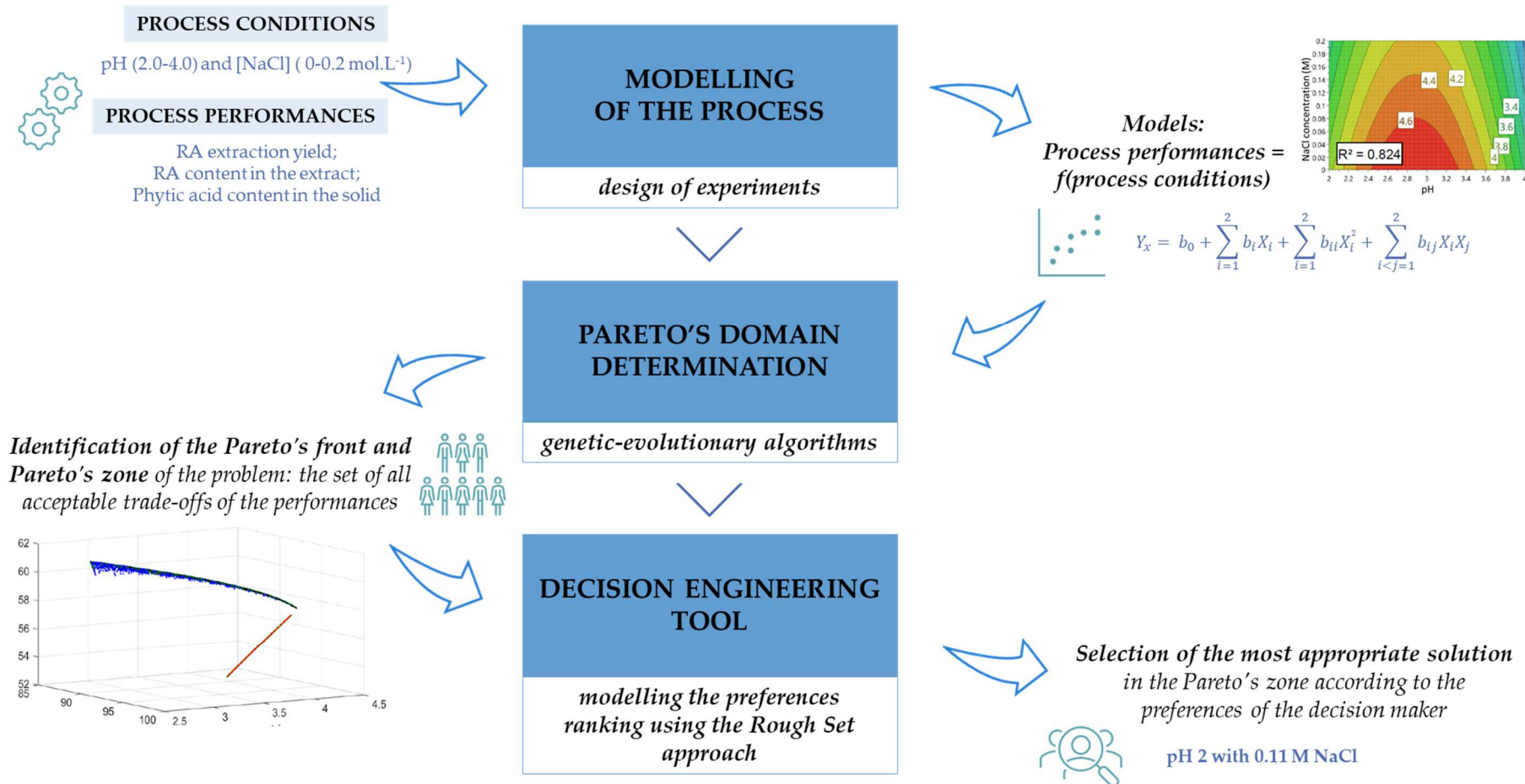
Finding optimal solutions with antagonist **process performance indicators** usually requires multicriteria decision-making (MCDM) optimization. This method increases the reliability of the choice of extraction conditions by giving clear and straightforward decision rules. Many MCDM methods were reported (Wątróbski et al. 2019). Muniglia et al. (2004) proposed an approach **involving** Pareto's domain determination and ranking by **Pareto's domain determination and then ranking, a method applicable to several bioprocesses.** The approach includes a DoE step to get information about the process system and polynomial models to

reliably describe the variation of each **process performance indicators** as a function of the process conditions. Then, the simulation models are implemented in a real encoding diploid genetic algorithm exploiting the Pareto's domination concept to generate the Pareto's front and domain. **In a final step of the method, the solutions belonging to the Pareto's domain are ranked by Decision Engineering.** The decision maker can eventually choose the best trade-off from its preferences. To date, such optimization approach has never been applied to valorize both RA and the remaining solid residue. In this paper, we therefore implemented this MCDM approach and analyzed its effectiveness to rationally identify the optimal conditions of selective RA extraction coupled with the production of a high-quality solid residue.

## **2. Methodology**

The approach used for the multicriteria decision-making (MCDM) optimization is depicted on Figure 1. The methodology includes three steps: (i) the process modelling (calculation of target **process performance indicators** as a function of process conditions); (ii) the determination of the Pareto's domain (identification of all acceptable trade-offs of **process performance indicators**); and (iii) the application of the decision-making tool (selection of the most appropriate trade-off and the corresponding process conditions). Each step is detailed below.





**Figure 1.** Methodology of multicriteria decision-making optimization applied for the identification of optimal condition of selective rapeseed albumin extraction coupled with the production of a high-quality solid residue.

## 2.1. Design of experiments

DoE was used to obtain the correlation models for calculating **process performance indicators** as a function of extraction conditions. The models were second-degree polynomials, which allow an accurate representation of non-linear systems. Table 1 presents the two process conditions (*i.e.*, extraction pH and NaCl concentration), as well as the three **process performance indicators** studied. The other process conditions (temperature, solid/liquid ratio, and extraction duration) were kept constant (Albe-Slabi et al. 2020a). Room temperature (20 °C) was applied to avoid protein denaturation, and to minimize energy consumption. A solid/liquid ratio commonly used for plant protein extraction (1/9; w/w) was applied allowing a trade-off between costs and efficiency of the production process. The extraction duration was of 60 min to be easily scaled-up. Three **process performance indicators** were implied: RA extraction yield ( $Y_1$ ); RA content in the extract ( $Y_2$ ); and the phytic acid content in the remaining solid residue ( $Y_3$ ). The protein content in the remaining solid residue was not included in the DoE because it can be easily deduced from mass balance with the initial content in the studied meal and the **process performance indicators**  $Y_1$  and  $Y_2$  (considering no mass loss).

**Table 1.** Process conditions and **process performance indicators** studied for modelling of the selective rapeseed albumin extraction.

Variables	Coded	Range
<i>Process condition</i>		
pH	$X_1$	2.0-4.0
NaCl concentration	$X_2$	0-0.2 mol.L <sup>-1</sup>
Variables	Coded	Desired optimization
<i>Process performance indicators</i>		
RA extraction yield (%)	$Y_1$	Maximization
RA content in the extract (%)	$Y_2$	Maximization

A central composite face centered design was used. The matrix consisted of 11 experiments (8 combinations and 3 replicates at the central point; Table S1). The polynomial equations describing each process performance ( $Y_x$ , Equation 1) were expressed with both process conditions ( $X_1$  and  $X_2$ ) and intercept ( $b_0$ ), linear ( $b_i$ ), quadratic ( $b_{ii}$ ) and interaction ( $b_{ij}$ ) coefficients:

$$Y_x = b_0 + \sum_{i=1}^2 b_i X_i + \sum_{i=1}^2 b_{ii} X_i^2 + \sum_{i < j=1}^2 b_{ij} X_i X_j \quad (1)$$

MODDE 12.0.1 software (Umetrics, Sartorius Stedim Biotech, Sweden) was used to fit the experimental data to the polynomial equations with adapted transformation if necessary. The regression coefficients were obtained by multivariable regression. An ANOVA was used to evaluate the statistical significance of these coefficients (p-value < 0.05). Final correlation models were obtained by suppressing the non-significant terms (p-value > 0.05). In the case where the coefficient of linear effect was non-significant, but at least one of the coefficients of cubic effect or interaction was significant, both coefficients were kept in the model. The coefficient of determination ( $R^2$ ), the relative standard deviation (RSD), the reproducibility and the lack of fit were evaluated using the MODDE software to assess the robustness of each model ( $Y_1$  and  $Y_2$ ).

## 2.2. Pareto's domain determination

The second step of the MCDM optimization is to obtain of all possible Pareto-efficient solutions using the domination concept. To do this, a routine developed internally upon Matlab software (MathWorks, Natick, Massachusetts, USA) using a genetic-evolutionary algorithm was applied (Muniglia et al. 2004). **This optimization tool is meant to identify the**

Pareto front for both simple and complex problems, *i.e.* with millions of possible solutions, in which case a genetic algorithm (or any stochastic algorithm) is needed for optimization.

This algorithm was based on the principles of biological evolutionary processes and used them as rules for optimization problems. The diploid version of the genetic-evolutionary algorithm was used since it was more efficient and faster than the haploid version (Fonteix et al. 1995). The real encoding diploid genetic algorithm used in this study has already been well described previously (Massebeuf et al. 1999; Perrin et al. 1997; Viennet et al. 1996).

In the present work, optimization of the above mentioned three antagonist **process performance indicators** (RA extraction yield ( $Y_1$ ); RA content in the extract ( $Y_2$ ); phytic acid in the remaining solid residue ( $Y_3$ )) was implemented. The objective function corresponded to the maximization of  $Y_1$  and  $Y_2$  and the minimization of  $Y_3$ . In a large population, each set of extraction conditions ( $X_1$  and  $X_2$ , *i.e.*, a combination of pH and NaCl concentration) represented an individual. All individual (pH and NaCl combination) were compared in pairs to determine if a given individual is dominated (*i.e.*, **another one has equivalent process performance indicators and at least one better process performance indicator**). **Although the number of possible solutions/individuals is small, a large population (20 000 individuals) was used in this study to ensure that the whole experimental domain is explored. Since computation time is small due to a simple mathematical model for extraction process, the size of the population is not limiting.** The algorithm thus resulted in a set of non-dominated solutions (all acceptable trade-offs of the **process performance indicators**) which can be represented either in the space of extraction conditions (Pareto's zone) or in the space of **process performance indicators** (Pareto's front).

### 2.3. *Decision Engineering tool*

The last step is the selection of the most appropriate solution according to the preferences of the decision maker using a decision engineering tool.

This consisted in modelling the preferences of the decision-maker and applying them to the whole Pareto's front to rank the **process performance indicator** set. In this approach, one sample of five **process performance indicator** sets were selected from the Pareto's front (preferably far from each other) randomly. These **process performance indicator** sets need to be manually ranked (from the most to the least preferred) by an expert called the decision-maker.

Then, the Rough Set approach was implemented as previously described (Huang, 1992; Thibault et al. 2003). The ranking was analyzed to determine if some preference rules or non-preference rules could explain the proposed ranking (Pawlak, 1982). To do this, the sets of **process performance indicators** were compared in pairs for each **process performance indicator**. No range of indifference (*i.e.*, measurement precision) for any of the results was established by the expert. The rules were coded in binary, as: 1 is attributed to one preferred **process performance indicator** higher than the same **indicator** of a less preferred set, otherwise, 0 is attributed to the **indicator**. As an example, if the set of **performance indicators** [ $Y_1= 55.5\%$ ;  $Y_2= 97.9\%$  and  $Y_3=3.6\%$ ] was preferred to the one [ $Y_1= 60.1\%$ ;  $Y_2= 91.3\%$  and  $Y_3=3.3\%$ ], it resulted in the preference rule 0-1-0, and the non-preference rule 1-0-1 ( $Y_3$  being minimized). **If the same rule existed as both a preference and a non-preference (due to the random and manual ranking selection of experts), this one was removed. If the rules of preference and non-preference existed, then they were applied to the set of process performance indicators of the whole Pareto's front and comparison in pairs was performed.** A score was thus assigned to each trade-off of **performance indicators** depending on how

many times it would be preferred (+1) or non-preferred (-1) to other trade-offs of the Pareto's front. Finally, each trade-off of **process performance indicators** in the Pareto's front was ranked relative to their final score. The ranking of solutions can be divided (in the top 10%, between 10 and 30%, 30 and 70%, and 70 and 100%) and shown with different colors. The best combination of **performance indicators** was selected in the top 10% ranked Pareto's solution.

### **3. Material and methods**

#### **3.1. Chemicals**

Hydrochloric acid (HCl, CAS 7647-01-0) was purchased from Carlo Erba (Val-de-Reuil, France). Sodium chloride (NaCl, CAS 7647-14-201), sodium hydroxide pellets (NaOH, CAS 1310-73-2) ethylenediaminetetraacetic acid (EDTA, CAS 6381-92-6) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Tris(hydroxymethyl)aminomethane (Tris, CAS 77-86-1), iron (III) chloride (FeCl<sub>3</sub>, CAS 7705-08-0), sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>, CAS 7757-82-6), 5-sulfosalicylic acid hydrate (CAS 304851-84-1) was from Fisher Scientific (Hampton, USA). All solvents (water and acetonitrile) were of analytical grade and purchased from Fisher Scientific (Hampton, VA, USA). Ultra-pure water ( $\geq 18.2$  M $\Omega$ ) was generated by a system Purelab Ultra from Elga (High Wycombe, UK).

#### **3.2. Selective rapeseed albumin extraction and purification**

Rapeseed albumins (RA) were extracted from a cold-pressed rapeseed meal provided by Olead (Pessac, France) as described by Nioi et al. (2012) with slight modifications. The content of the proteins and phytic acid in the initial meal were around 37.4% (w/w; N $\times$ 6.25) and 6.8% on dry matter basis, respectively.

The solid/liquid extraction was carried out by mixing the rapeseed meal with water at a 1/9 solid/liquid ratio (w/w) at 20 °C. NaCl was added at a given concentration (0-0.2 mol.L<sup>-1</sup>). Then, the pH was adjusted to given values (2.0- 4.0) using 1 mol.L<sup>-1</sup> HCl solution. The mixture was stirred at 600 rpm during 60 min. Then, the mixture was centrifuged (15 000 × g, 15 min, 20 °C) and filtered through a cellulose paper of 0.2 µm pore-size membrane from Fisher Scientific (Pittsburgh, Pennsylvania, USA). After, the extract was clarified by microfiltration using a 0.22 µm membrane cut-off (0.1 m<sup>2</sup> Pellicon XL membrane, Millipore, Burlington, MA, USA) on a ProScale system (Millipore, Burlington, MA, USA) at a volumetric reduction factor (VRF) of 10. The remaining solid residue was collected and freeze-dried for further characterization.

RA obtained by the optimal set of extraction conditions was purified by tangential ultrafiltration in diafiltration mode to reach the isolate grade (*i.e.*, protein purity >90% on a dry matter basis). The diafiltration was performed using an ultrafiltration system (Äkta Flux 6, GE Healthcare, Chicago, IL, USA) and a 3 kDa cut-off 4 800 cm<sup>2</sup> hollow fiber cartridge (GE Healthcare, Chicago, IL, USA). The conditions were set at a 2 L.min<sup>-1</sup> feed flow, a transmembrane pressure of 1.5 bars and room temperature. The extract was washed with 5 diavolumes of 0.5 mol.L<sup>-1</sup> NaCl and then, 4 diavolumes of ultrapure water to remove salts. The final ultrafiltration retentate was collected and freeze-dried (LTD type GZL-0.5, Shanghai Pharmaceutical Machinery Co., Shanghai, China).

### 3.3. Analytical methods

#### 3.3.1. Kjeldahl method

The analyses for determining the total nitrogen content in sample were achieved according to the official AOAC 991.20 Kjeldahl method procedures (1995). For this purpose, 0.5-2 mL

of liquid sample or 30 mg of solid sample were mixed with 4 mL of 96% (v/v) sulfuric acid (Sigma-Aldrich, Saint-Louis, MO, USA) and approximately 10 mg of catalyst Cu-Se (AppliChem, Gatersleben, Germany). The mineralization step was carried out at 450 °C for 135 min in a digestion flask (Büchi SpeedDigester K-439, Rungis, France). The distillation step (Kjelflex K-360, also from Büchi) was done with 32% sodium hydroxide (w/v) (VWR, Radnor, PE, USA) and 3% (w/v) boric acid (Sigma-Aldrich, Saint-Louis, MO, USA). The titration was done against 0.01 mol.L<sup>-1</sup> HCl solution (Honeywell, Morris Plains, NJ, USA) in a Titrino Plus 877 (Metrohm, Herisau, Switzerland). A blank was also analyzed with all reagents without the initial sample. A nitrogen-to-protein conversion factor of N×6.25 was used. All measurements were performed in triplicate and a mean value was calculated.

### 3.3.2. Albumin content in extract

RA was quantified in the extracts by size exclusion high performance liquid chromatography (SE-HPLC) according to the method developed by Defaix et al. (2019). A Biosep-SEC-s2000 (300 × 7.8 mm, 5 µm) from Phenomenex (Torrance, CA, USA) was used, connected to a Shimadzu model LC20 system (Shimadzu Corporation, Kyoto, Japan). An isocratic elution was used to separate the samples at 0.6 mL.min<sup>-1</sup> with a water/acetonitrile/trifluoroacetic acid (TFA): 54.9/45/0.1 (v/v) solvent. A 5 µL of filtered sample were injected onto the column and the elution was performed during 30 min. The UV signal was recorded at 214 nm. Data were collected and treated using LabSolutions software (Shimadzu Corporation, Kyoto, Japan).

RA content in the extract is the proportion of this protein fraction in relation to the total soluble proteins in the extract (*i.e.*, albumins and globulins). This parameter was determined as below:



$$C_{ALB} = \frac{A_{ALB}}{A_{GLOB} + A_{ALB}} \times 100 \quad (\text{Eq. 2})$$

where  $C_{ALB}$  is the albumin content in the extract;  $A_{ALB}$  and  $A_{GLOB}$  are the peak surfaces at 214 nm corresponding to the globulins and albumins, respectively.

The RA extraction yield was calculated according to the following equation:

$$Y_{ALB} = \frac{C_{Pe} \times V_e \times C_{ALB}}{C_{Pm} \times m_m \times 35\%} \times 100 \quad (\text{Eq. 3})$$

where  $Y_{ALB}$  is the RA extraction yield (%);  $C_{Pe}$  and  $C_{Pm}$  are the protein concentrations measured by the Kjeldahl method in the crude extract ( $\text{g}\cdot\text{L}^{-1}$ ) or in the starting rapeseed meal ( $\text{g}\cdot\text{g}^{-1}$ ), respectively;  $V_e$  is the volume of the crude extract (L);  $C_{ALB}$  is the RA content in the extract (%; Eq. 2) and  $m_m$  is the weight of the meal on a dry matter basis (g). For the calculations, a mean value of the initial RA content in rapeseed meal of 35% was assumed (von der Haar et al. 2014; Wanasundara, 2011).

### 3.3.3. Phytic acid content in the solid residue

Phytic acid content in the solid residue remaining after the protein extraction was determined by the method of Garcia-Esteva et al. (1999) slightly adapted. First, about 250 mg of solid sample was mixed with  $0.4 \text{ mol}\cdot\text{L}^{-1}$  HCl + 10% (w/v)  $\text{Na}_2\text{SO}_4$  solution and stirred during least 2 hours. After centrifugation ( $15\,000 \times g$ , 15 min,  $20 \text{ }^\circ\text{C}$ ), the supernatant was recovered, filtered using a  $0.22 \text{ }\mu\text{m}$  size membrane and mixed with equal volume of the following solutions:  $20 \text{ mmol}\cdot\text{L}^{-1}$   $\text{FeCl}_3$ , 20% (w/v) sulfosalicylic acid, and  $0.4 \text{ mol}\cdot\text{L}^{-1}$  HCl solutions. The mixture was then heated at  $100 \text{ }^\circ\text{C}$  during 20 min and after cooling and centrifuging (the same parameters as above), the supernatant was recovered. The resulting

precipitate was washed three times with ultra-pure water. Then, 20 mL of mixture was placed in a water bath heated at 80 °C and titrated against 2 mmol.L<sup>-1</sup> EDTA solution. The equivalent volume was reached when the solution changed color from burgundy to yellow green. Finally, the mass of phytic acid was determined and the phytic acid content was expressed relatively to the dry matter of the solid sample. All measurements were performed in triplicate and a mean value was calculated.

### 3.4. Albumin isolate characterization

#### 3.4.1. Purity and solubility

The purity and solubility of the RA isolate obtained were measured using the Kjeldahl method (*section 3.3.1*) and expressed on dry matter basis (N×6.25). For measurement of the protein solubility, 20 mL of the RA were dissolved in deionized water at a concentration of 5 g.L<sup>-1</sup> at room temperature. The pH was adjusted to given values (3.0, 5.0, 7.0 and 9.0) by adding 0.1 mol.L<sup>-1</sup> HCl or NaOH. The pH of mixture was kept constant (±0.05) during 30 min under agitation and then centrifuged (11 000 × g; 15 min; 20 °C). The supernatant was recovered, and the concentration of soluble proteins was then measured using the Kjeldahl method. The solubility was calculated as the ratio of the mass of the proteins in the supernatant to the mass of the proteins in the initial solution.

#### 3.4.2. Color analysis

The color of the RA isolate obtained was determined in aqueous solution with a Lovibond PFX195 (Tintometer, Amesbury, UK). A 1% (w/w) solution of protein powder was prepared in 0.5 mol.L<sup>-1</sup> phosphate buffer, pH 7.0. The color of the sample was expressed according to the CIELAB uniform space scale, with the term “L” related to the quantity of black/white;

the term “a” to green/red; and the term “b” to blue/yellow. All measurements were performed in triplicate and a mean value was calculated.

#### 3.4.3. Foaming properties

The foaming properties of the optimal RA isolate were assessed by the methods adapted from the reported works of Vioque et al. (2000). The protein isolate was dissolved in 0.5 mol.L<sup>-1</sup> sodium phosphate buffer, pH 7.0 at a final concentration of 1% (w/v, N×6.25). A 20 mL of the protein solution were mixed at 10 000 rpm at room temperature for 5 min using an Ultra-Turrax® T25 digital homogenizer from IKA (Staufen im Breisgau, Germany). The foaming capacity was expressed (in %) as a ratio between the obtained foam volume and the volume of initial protein solution before mixing. The foaming stability was calculated (in %) as the volume of the remaining foam after 120 min (room temperature).

#### 3.4.4. Emulsifying properties

The emulsifying properties of the optimal RA isolate were assessed by the methods adapted from the reported works of Vioque et al. (2000). For this purpose, 5 mL of aliquot of 5% (w/v, N×6.25) protein solution in 0.5 mol.L<sup>-1</sup> sodium phosphate buffer, pH 7.0 were vigorously stirred with 2.5 mL of commercial rapeseed oil at 10 000 rpm at room temperature during 30 s using an Ultra-Turrax® T25 digital homogenizer from IKA (Staufen im Breisgau, Germany). After this time, 2.5 mL of oil were added again and stirred during 90 s at 10 000 rpm. The formed emulsion was centrifuged at 1 100 × g during 5 min at 20 °C. The emulsified layer was recorded. The emulsifying capacity (in %) was calculated as a ratio of the volume of the emulsified layer remaining after centrifugation and the volume of the mixture before centrifugation. For evaluation of the emulsion stability against temperature, the mixture obtained after centrifugation was placed in a water bath heated at 85 °C during

15 min. After cooling at room temperature, they were centrifuged again at  $1100 \times g$  for 5 min. The emulsion stability was calculated (in %) as a ratio of the emulsified layer before and after heating.

#### 3.4.5. Statistical analysis

All experiments were performed at least in triplicate ( $n=3$ ) and data were expressed as means with standard deviation ( $\pm$  SD). The statistical analysis of the models was carried out with the DoE software MODDE 12.0.1 software (Umetrics, Sartorius Stedim Biotech, Sweden) as described in section 2.1. The other analyses were performed using the freeware R (3.4.1.) and the Analysis ToolPak of Excel (Microsoft Office, Redmond, Washington, USA).

## 4. Results and discussion

### 4.1. Extraction process modelling

Table 2 shows regression coefficients of the obtained models for the RA extraction yield ( $Y_1$ ), the RA content in the extract ( $Y_2$ ) and the phytic acid in the remaining solid residue ( $Y_3$ ). The significance of terms was assessed with an analysis of variance (ANOVA), and non-significant terms were removed to improve the reliability of the models. Transformation was applied when necessary. The intercept, linear, quadratic and interaction coefficients are presented for the three models.

**Table 2.** Regression coefficients of the predicted second-degree polynomial models for the RA extraction yield ( $Y_1$ ), the RA content in the extract ( $Y_2$ ) and the phytic acid in the remaining solid residue ( $Y_3$ ) and ANOVA analysis results.

<b>Variables</b>	<b>RA extraction yield</b>	<b>RA content in the extract</b>	<b>Phytic acid content in the remaining solid residue</b>
<i>Transformation</i>	<i>Logarithmic</i>	<i>Logarithmic</i>	-

<i>Coefficients</i>			
<b>Intercept</b> ( $b_0$ )	-1.48	-0.48	-5.04
<b>Linear</b> ( $b_i$ )			
<i>pH</i>	-0.07	0.30	6.87
<i>NaCl concentration</i>	-1.32	-	-2.99
<b>Quadratic</b> ( $b_{ii}$ )			
<i>pH* pH</i>	-	-0.11	-1.19
<i>NaCl * NaCl</i>	-	-	-
<b>Interaction</b> ( $b_{ij}$ )			
<i>pH* NaCl</i>	0.54	-	-
<i>Analysis of variance</i>			
<b>R<sup>2</sup></b>	0.847	0.940	0.824
<b>Q<sup>2</sup></b>	0.563	0.896	0.674
<b>Reproducibility</b>	0.852	0.982	0.667
<b>RSD</b>	0.022	0.078	0.387
<b>Regression (p-value)</b>	0.003	<0.001	0.005
<b>Lack of fit (p-value)</b>	0.418	0.200	0.695

Table 2 also displays the statistical results of the analysis of variance (ANOVA test). It appeared that the probabilities for the regression of all responses were highly significant at 95% (p-value of the regression < 0.05). This indicated that all models obtained were similar with the experimental data and statically good. Furthermore, the models for “RA extraction yield” prediction ( $R^2 = 0.847$ ), “RA content in the extract” prediction ( $R^2= 0.94$ ) and for “Phytic acid content in the remaining solid residue “prediction ( $R^2= 0.824$ ) fitted well the experimental data. Previous studies on plant protein extraction of mung bean, pigeon pea, lentil, and sunflower albumin, have reported coefficients of determination ( $R^2$ ) of 0.904 (Du et al. 2018), 0.775 (Mizubuti et al. 2000), 0.81 and 0.93 (Jarpa-Parra et al. 2014) and from 0.802 to 0.981 (Albe-Slabi et al. 2020b), respectively. The  $Q^2$  (0.56-0.90) and reproducibility values (0.67-0.98) expressed useful models to well predict new data. Error analysis showed that the

probabilities for lack of fit were not significant at 95% (p-value of the regression > 0.05). Thus, the chosen second-degree polynomial models allowed good prediction in the considered domain and were well adapted to satisfactorily describe the process of protein extraction. This was also the case in the studies of Albe-Slabi et al. (2020a) and Pickardt et al. (2009) for the optimization of sunflower protein extraction.

Among all **process performance indicators**, the phytic acid content in the remaining solid residue was the most influenced by extraction conditions. The RA content in the extract was only affected by the pH condition. The quadratic terms of NaCl concentration were not significant ( $p > 0.05$ ) for all **performance indicators**. Interaction between pH and NaCl concentration was only found for the RA extraction yield model. Hence, the proposed methodology of multicriteria decision-making optimization should be applied to assist the experimenter/industrial to identify all possible Pareto-efficient solutions among the **process performance indicators**, and thus the process conditions to apply.

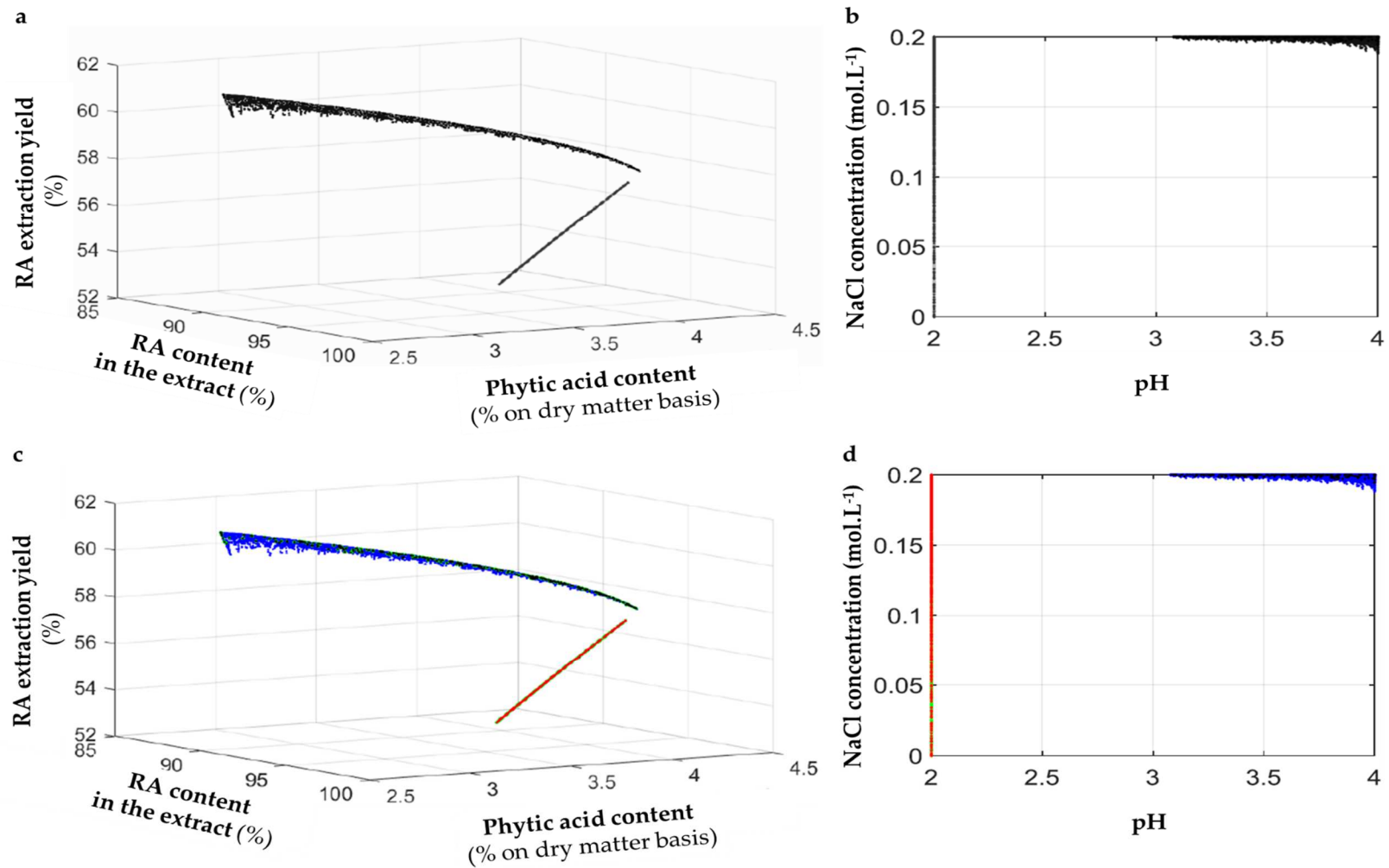
## 4.2. Multicriteria decision-making optimization

### 4.2.1. Pareto's domain determination

The initial population of 20 000 individuals (combination of process conditions) led to 17 469 non-dominated points using the diploid genetic-evolutionary algorithm. Each non-dominated point corresponded to an acceptable trade-off of the three **process performance indicators** studied. Due to Pareto's domination, the increase of one **indicator** in this set would automatically alter another **indicator**. This set of non-dominated solutions obtained were represented on the Pareto's front (the space of **process performance indicators**) and on the Pareto's zone (the space of extraction conditions), as presented on Figure 2a and b, respectively.

Interestingly, two zones were distinguished on the Pareto's domain. A first one composed of 13 598 non-dominated points was located at pH 2.0 with salt from 0 to 0.2 mol.L<sup>-1</sup> NaCl. It corresponded to very high RA contents in the extract (around 98%), but moderate RA extraction yields (53.7 – 57.8%) and medium or even high contents of phytic acid in the residue (3.3 – 3.9%). This high selectivity of the extraction of RA demonstrated at pH 2.0 was consistent with the previous work of Nioi et al. (2012), but the latter did not consider the effect of NaCl concentration. This was also in accordance with the observations of Albe-Slabi et al. (2020b) on the extractability of albumins from sunflower meal who showed higher albumin content at low pH and varying NaCl concentration.

A second zone was made up of 3 times fewer non-dominated points (3 880). This one was located between pH 3.1 and pH 4.0 with high NaCl concentration (0.2 mol.L<sup>-1</sup>). In that area, the RA extraction yields were close to maximum values (58 – 61%), but with lower RA content in the extract (88.3 – 95.6%) and a large range of phytic acid in the residue from low to high values (2.75 – 4.2%). These conditions were closed to those reported (pH 3.0; 0.15 mol.L<sup>-1</sup> NaCl) on the RA extraction by Perera et al. (2016).



**Figure 2.** Pareto's front (a) and Pareto's domain (b) of all acceptable solutions for the optimization of the selective extraction of rapeseed albumins (RA) with the by-production of high-quality solid residue. Ranked Pareto's front (c) and ranked Pareto's domain (d) according to the decision-maker preferences and the set of rules obtained with the Rough Set approach. Colors describe the ranking of the solutions: ranked in the top 10% of all solutions (red), ranked between 10 and 30% (green), ranked between 30 and 70% (black) and ranked between 70 and 100% (blue).



These interesting results meant that the best trade-off between **the performance indicators of extraction process** has to be searched by either tuning the NaCl concentration at pH 2.0 or tuning the pH from 3.1 to 4.0 at 0.2 mol.L<sup>-1</sup> NaCl. Moreover, these observations demonstrated that the high selectivity of RA extraction was to the detriment of the productivity of the process (RA extraction yield) and the quality of the solid residue. The results of this optimization step showed competing objectives of the extraction process, making it more difficult to choose an optimal solution for all the objectives. Hence, the knowledge of a decision-maker on the studied process should be used in the Rough Set approach to find the best rational trade-off among all non-dominated solutions.

#### 4.2.2. Decision Engineering tool

The Pareto's domain gave useful information on the feasible trade-offs of the considered process. However, it was not possible to rank the 17 469 solutions. The first step of the Rough Set approach applied was to present a sample of different acceptable trade-offs to the decision-maker, *i.e.*, a human expert of the RA extraction process. Table 3 shows the sample presented to the decision-maker with five selected **process performance indicators** combinations from all possible trade-offs. These sets were then ranked according to the decision-maker preferences, from the most preferred (1) to the least preferred (5). As it can be seen, the expert of the process expressed preferences for combinations with high RA content in the extract and moderate RA extraction yield and phytic acid content in the residue. In this work, it was chosen to establish no indifference range for any **performance indicator**. At this stage, the contribution of the decision-maker was accomplished.

**Table 3.** Ranked sets of **process performance indicators** according to the decision-maker preferences for the selective extraction of rapeseed albumins with the by-production of high-quality solid residue and corresponding process variable values.

Rank*	RA content in the extract (Y <sub>2</sub> ; %)	Phytic acid content in the remaining solid residue (Y <sub>3</sub> ; %)	RA extraction yield (Y <sub>1</sub> ; %)	pH	NaCl concentration
1	97.9	3.59	55.5	2,00	0,112
2	98.2	3.92	57.8	2,00	0,000
3	97.7	3.35	53.8	2,00	0,193
4	93.9	3.89	59.0	3,42	0,200
5	91.4	3.34	60.1	3,75	0,199

\*From the most preferred to the least preferred combination according to the decision-maker

From the ranked sets of process performance indicators, three rules of preference and three rules of non-preference were defined. Table 4 presents these final rules based on previously ranged indicators (Table 3) after elimination of the same rules of preference and non-preference. Then, these rules were used to rank all the non-dominated solutions of the Pareto's domain. A ranking score was therefore assigned to each solution.

**Table 4.** Preference and non-preference rules for the sample of the ranked process performance indicators for the selective extraction of rapeseed albumins with the by-production of high-quality solid residue.

	RA content in the extract (Y <sub>2</sub> )	Phytic acid content in the remaining solid residue (Y <sub>3</sub> )	RA extraction yield (Y <sub>1</sub> )
<b>Preference rules</b>	1	0	0
	1	1	0
	0	1	0
<b>Non-preference rules</b>	0	0	1
	0	1	1
	1	0	1

Figure 2c and d represent the ranking of the 17 469 acceptable solutions with different colors on the Pareto's front and on the Pareto's zone, respectively. Red dots represent the top 10% of solutions with the highest-ranking scores. Other colors were attributed to the solutions ranked between 10 and 30% (green dots), between 30 and 70% (black dots), and between 70 and 100% (blue dots). The optimal solution should therefore be selected in the top 10% of ranked solutions (red dots). These solutions appeared in the first area of the Pareto's domain previously observed at pH 2.0 with salt from 0 to 0.2 mol.L<sup>-1</sup> NaCl. This was expected since the decision-maker preferred combinations with high RA content in the extract and moderate RA extraction yield and phytic acid content in the residue (Table 3). It resulted in a red distinct linear band of points on the Pareto's front, oriented linearly along the axis of the phytic acid content and the RA extraction yield, which proved the preference of a high RA selectivity over a high extraction yield and a low phytic acid content.

Among these solutions, the best one has to be chosen for identifying the extraction conditions that will be applied for the process. One combination of **process performance indicators** was proposed as it had the highest-ranking score (6 248) of all ranked solutions. The **process performance indicators** and conditions of this optimal solution identified are presented in Table 5. The combination of the **performance indicators** proposed was in accordance with the decision-maker. The solution pointed out a medium NaCl concentration (0.11 mol.L<sup>-1</sup>) which is in accordance with the constraints of industrial implementation. Also, this optimal solution exhibited high reliability because it was surrounded by other solutions also ranked in the top 10%. This would ensure similar outcomes in case of small variations during the process. Hence, the optimal solution proposed was satisfying and accepted, and no additional preferences were needed. To our knowledge, it was the first time that this

combination of extraction conditions was highlighted for the selective RA extraction with the by-production of high-quality solid residue.

**Table 5.** Optimal process conditions and **process performance indicators** identified for the selective extraction of rapeseed albumins with the by-production of high-quality solid residue.

<i>Process condition</i>	<b>Optimal solution identified</b>
pH	2.0
NaCl concentration	0.11 mol.L <sup>-1</sup>
<i>Process performance indicator</i>	
RA extraction yield	55.5 %
RA content in the extract	97.9 %
Phytic acid content in the solid residue	3.58 %

#### 4.3. Characterization of rapeseed albumins obtained upon optimal conditions

The extraction process was implemented upon optimal conditions highlighted (Table 5) to produce a RA isolate by ultrafiltration. The purity of the final protein product was 91.6% on dry matter basis (measured with the Kjeldahl method, factor N×6.25), corresponding to the isolate grade (Wanasundara, 2011). The optimized selective extraction process showed an albumin yield of  $48.6 \pm 3.1\%$ , an albumin content in the extract of  $97.3 \pm 0.61\%$ . The phytic acid content in the remaining solid residue was  $4.6 \pm 0.5\%$ . This corresponded to a slight underestimation of the phytic acid in the solid residue and overestimation of the RA extraction yield. The discrepancy observed with phytic acid content can be explained by a bias in the equivalent volume and thus the phytic acid content. Nevertheless, the experimental results remained acceptable for such a complex process. These experimental results therefore validated the optimization approach applied. In addition, the high RA selectivity desired by the process expert was experimentally validated. Also, the

nitrogen/protein content in the remaining solid residue was 35.6% on dry matter basis (measured with the Kjeldahl method, factor  $N \times 6.25$ ). The optimal extraction therefore allowed for the by-production of a high-quality solid residue with great potential for feed applications.

The quality of the RA isolate thus obtained was further characterized for food applications.

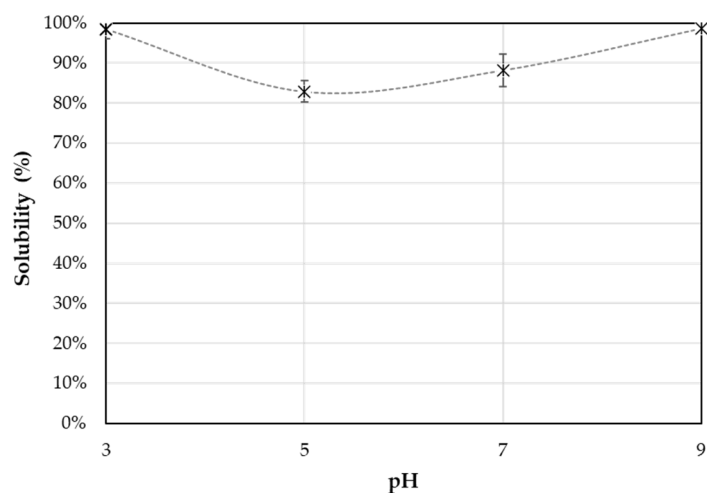
**The isolate produced had a pleasant slight nutty flavor.** The color of the isolate in solution at pH 7.0 was therefore assessed considering the CIELAB uniform space. The resulting index of lightness ( $L^*$ ) and the two color coordinates ( $a^*$  and  $b^*$ ) were  $92 \pm 2.7$ ,  $-4.22 \pm 0.2$ , and  $24.3 \pm 0.54$ , respectively. These results corresponded to a light creamy powder product, which is very interesting compared to the recent study of Fetzer et al. (2019). Indeed, it showed much darker color of products composed of total rapeseed proteins, with an index of lightness  $L^*$  around 50. Since the color of the final product is usually a primordial quality criterion in food formulations, these results proved the great potential of RA isolate use.

The solubility in aqueous medium of a protein product is also important since it conditions the other techno-functional properties (Moure et al. 2006). The solubility has to be high enough to allow interesting food-based applications. The processes for obtaining protein products (extraction and purification) can have a strong influence on the solubility of the final product. The solubility of the optimal RA isolate obtained was measured with the Kjeldahl method, from pH 3.0 to 9.0 to cover a wide range of potential applications. The solubility measurements are exposed on Figure 3. High solubility was highlighted ( $> 83\%$ ) on all the pH range. The isolate obtained was totally soluble at pH 3.0 and 9.0. Hence, this high solubility of the optimal RA isolate can be very interesting for food formulations. Furthermore, it was exceptionally high when comparing to other plant proteins (Amagliani

et al. 2017; Ivanova et al. 2013; Tang et al. 2006) and was in accordance with previous works on rapeseed albumins (Cheung et al. 2015; Perera et al. 2016).

The techno-functional properties (emulsifying and foaming properties) of the RA isolate were also characterized for industrial applications. Foaming capacity of  $263.6 \pm 18\%$  and foaming stability (after 120 min) of  $66.6 \pm 0.5\%$  were measured. These good foaming properties agreed with the conclusions of various previous studies (Chabanon et al. 2007; Wanasundara et al. 2016) and were explained by the low molecular weight of RA that facilitate the molecule diffusion and the quick adsorption to the interfaces (Krause & Schwenke, 2001). The RA isolate was characterized with an emulsifying capacity of  $32.7 \pm 2.54\%$  and total emulsifying stability (100%). The moderate emulsifying capacity can be due to the globular conformation of RA limiting the exposition of hydrophobic amino acids at the surface of the proteins. The emulsifying capacity observed was equivalent to that reported in the study of Chabanon et al. (2007), but they reported a 2 times lower emulsifying stability. The excellent stability observed in our study was also higher than the one measured in the works of Wu & Muir (2008).

In conclusion, the RA isolate produced upon optimal conditions of selective extraction (pH 2 and  $0.11 \text{ mol.L}^{-1}$ ) exhibited excellent solubility in aqueous water over a wide range of pH, promising emulsifying properties and good foaming properties, as well as an adequate color powder. These characterization results thus validated the optimal solution proposed by the applied MCDM optimization and highlighted the high potential for RA valorization in industrial food-based applications.



**Figure 3.** Solubility of rapeseed albumin isolate (RA, 5 g.L<sup>-1</sup>) as a function of pH ranging from 3.0 to 9.0 at 20 °C measured by the Kjeldahl method. Values are means (n = 3) and the error bars are the standard deviations.

## 5. Conclusion

This study has considered the multicriteria optimization of selective albumin extraction from a rapeseed cold-pressed meal based on the Rough Set approach. For the first time, the optimization strategy of this extraction process considered both competing **process performance indicators** of RA extraction and quality of the solid residue remaining from this process. The results of the Pareto's domain determination brought to light the competing objectives of the extraction process. Indeed, it was shown that the high selectivity of RA extraction was to the detriment of the productivity of the process (RA extraction yield) and the quality of the remaining solid residue. The decision engineering tool, based on the Rough Set approach, allowed identifying the best trade-off of extraction conditions at pH 2.0 and 0.11 mol.L<sup>-1</sup> NaCl. Based on these results, the selective extraction process of RA resulted in high selectivity (97.9% of RA in the extract), and good RA extraction yield (55.5%), with the production of a protein-rich solid residue (35.6% on dry matter basis) and reduced phytic acid content (3.58% on dry matter basis). The RA isolate obtained in the identified extraction conditions was light creamy colored, highly soluble over a wide range of pH, and

exhibited promising emulsifying properties and good foaming properties. Hence, the application of the proposed multicriteria optimization approach has been very useful in valuing both the RA and the solid residue of the process for food and feed applications, respectively, in an overall concept of circular development.



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