We are IntechOpen, the world’s leading publisher of Open Access books
Built by scientists, for scientists

5,600
Open access books available

138,000
International authors and editors

170M
Downloads

154
Countries delivered to

TOP 1%
Our authors are among the most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com
Chapter

Response Surface Methodology Applied to the Optimization of Phenolic Compound Extraction from *Brassica*

Valentin Reungoat, Morad Chadni and Irina Ioannou

Abstract

The response surface methodology (RSM) is a relevant mathematical and statistical tool for process optimization. A state of the art on the optimization of the extraction of phenolic compounds from *Brassica* has shown that this approach is not sufficiently used. The reason for this is certainly an apparent complexity in comparison with the implementation of a one-factor-at-a-time (OFAT) optimization. The objective of this chapter is to show how one implement the response surface methodology in a didactic way on a case study: the extraction of sinapine from mustard bran. Using this approach, prediction models have been developed and validated to predict the sinapine content extracted as well as the purity of the extract in sinapine. The methodology presented in this chapter can be reproduced on any other application in the field of process engineering.

Keywords: Response surface methodology, extraction, phenolic compounds, process engineering, biomass valorization

1. Introduction

Nowadays, bio-based molecules are more and more popular and used in everyday consumer products. Certain molecules such as phenolic compounds (PCs) are very appreciated for their biological activities which make it possible to fight against aging or to act as an antibacterial or anti-oxidant agent. Phenolic compounds are secondary metabolites of plants and are present in plant biomass as well as in agro-industrial by-products [1]. The latter are currently used in sectors with low added value such as methanization or animal feed. To provide additional value to its agro-industrial co-products, phenolic compounds could be extracted and concentrated [2]. For this, separation processes will have to be implemented and optimized. Thus, maximizing the extraction of phenolic compounds has become a topic of interest which would improve the profitability of crops and by-products resulting from their industrial transformation [3].

Many studies focus on maximizing extraction efficiency by optimizing using OFAT. This method, which seems simpler, is often either time consuming or leads to partial conclusions (e.g. no interpretation of the interactions between variables). Thus, to achieve such an optimization, it is recommended, if conditions allow it, to
use the response surface methodology. RSM is a mathematical and statistical tool for exploring the relationships between several explanatory variables - called factors - and one or more variables to be optimized, called response(s). RSM is particularly relevant when the response is suspected to evolve in a curved way.

In this chapter, we will focus on the application of RSM for optimizing the extraction of phenolic compounds from *Brassica*. In the first part, we propose a state of the art of the studies on this topic with an analysis of the main tools used to determine the optimal operating conditions for the extraction of phenolic compounds. In a second part, a case study based on the work of Reungoat et al. (2020) is presented [4]. This study focuses on the optimization of a sustainable extraction process to improve the recovery (yield and purity) of sinapine from mustard bran. Sinapine has biological activities however, its first interest is the degradation product of its hydrolysis: the sinapic acid. It has been shown that providing bio-based sinapic acid is very relevant in various application fields [5]. Indeed, this platform molecules can be used for the chemo-enzymatic synthesis of various molecules such as an anti-UV agent [6, 7], a non-endocrine disruptive antiradical additive [8] and a bisphenol A substitute for polymer/resin synthesis [9]. The study will be detailed not from an application point of view but from a methodological point of view with the presentation of the different steps which led to obtaining optimum operating conditions of the extraction process.

2. State of the art on the optimization of PC extraction from *Brassica*

The studies reported in Table 1 deal with the optimization of the extraction process of phenolic compounds from Brassica. These all relate to the use of a design of experiments (DoE). OFAT optimization has been excluded.

Twenty papers have been identified on various raw materials belonging to *Brassica* (rapeseed, mustard, cabbage, broccoli, cauliflower). The extraction processes implemented are the most popular ones: conventional solvent extraction (CSE), ultrasound-accelerated extraction (UAE), microwave-accelerated extraction (MAE). One study deals with an extraction assisted by pulsed electric field (PEF) [13] and another with accelerated solvent extraction (ASE) [5].

The operating conditions the most often optimized are the extraction temperature, the solvent concentration in water, the solid-to-matter ratio, and the extraction time. Some specific conditions can also be investigated such as ultrasonic or microwave power when UAE and MAE are carried out.

The predicted responses are diverse whether they are measurement of individual phenolic content obtained by HPLC, total phenolic compounds (TPC), or content of total flavonoid (TFC), or antioxidant activity (AA) which can be measured by different methods (Table 2).

Most studies have used RSM to model and/or predict responses. A mixture design was also used to determine the composition of an extraction solvent from three pure solvents; a simplex centroid mixture was carried out [3]. Some studies model responses using first order polynomial equations. These models are obtained from factorial design of experiments [5, 15, 16]. Concerning the implementation of the RSM, the experimental design carried out are mainly Box–Behnken (BB) [8, 10, 13, 14, 17] and Central Composite (CC) [1, 6, 7, 9, 18–20]. We also found a D-optimal [4] and a full factorial [2]. However, these DoEs are rarely associated with RSM. The predictions made by RSM are associated with second order polynomial models.

Compared to all the studies that exist in the literature on the extraction of phenolic compounds from *Brassica*, only a small proportion uses RSM.
| Biomass                        | Extraction process | DOE | Factors                                                                 | Response                  | Model                               | Ref   |
|-------------------------------|--------------------|-----|-------------------------------------------------------------------------|---------------------------|-------------------------------------|-------|
| Mustard bran (*Brassica juncea*) | CSE                | CCF | • T (°C): 45, 60, 75                                                   | Sinapine yield, sinapine purity | Second-order polynomial             | [4]   |
|                               |                    |     | • E (%): 45, 70, 95                                                   |                           |                                     |       |
|                               |                    |     | • S/M (mL/g): 10, 20, 30                                              |                           |                                     |       |
| Rapeseed cake (*Brassica napus*) | CSE                | $2^3$ full factorial | • T (°C): 20, 30, 45, 60, 70                                         | TPC                       | Second-order polynomial             | [10]  |
|                               |                    |     | • E (%): 0.17, 43, 68, 85                                            |                           |                                     |       |
|                               |                    |     | • S/M (g/100 mL): 5, 8, 12.5, 17, 20                                   |                           |                                     |       |
| Mustard seeds (*Sinapis alba, Brassica nigra*) | CSE                | Simplex centroid mixture | Mixture of solvents: Water: 0,1/6, 1/2, 2/3,1 | TPC, AA | Quadratic or cubic regression | [11]  |
|                               |                    |     | Acetone: 0.1/6, 1/2, 2/3,1                                            |                           |                                     |       |
|                               |                    |     | Methanol: 0,1/6, 1/2, 2/3,1                                           |                           |                                     |       |
| Mustard bran (*Brassica juncea*) | CSE                | D-optimal | • T (°C): 30, 42.5, 55                                               | TPC                       | Second-order polynomial             | [12]  |
|                               |                    |     | • Time (h): 0.5, 3.25, 6                                               |                           |                                     |       |
|                               |                    |     | • E (%): 30, 65, 100                                                  |                           |                                     |       |
|                               |                    |     | • S/M (mL/g): 10, 30, 50                                               |                           |                                     |       |
|                               |                    |     | • Pretreatment: none, dried and defatted                               |                           |                                     |       |
| Canola meal (*Brassica napus*)  | Accelerated Solvent Extraction Factorial | | • Particle size (mm): 0.5, 1.                                          | TPC, TFC                  | First-order polynomial              | [13]  |
|                               |                    |     | • T (°C): 140, 160, 180                                               |                           |                                     |       |
|                               |                    |     | • Type of solvent: water, ethanol, methanol                            |                           |                                     |       |
|                               |                    |     | • Percentage of solvent in water (%): 30, 40, 60                      |                           |                                     |       |
| *Brassica oleracea* leaves | CSE | CC | • Time (h): 12, 24, 36, 48, 60                                          | TPA, TAA, AA              | Second-order polynomial             | [14]  |
|                               |                    |     | • Polarity of extracting solvents in terms of dipole moment (D): 0.0 (hexane), 4.40 (diethyl ether), 2.80 (ethylene acetate), 5.10 (methanol) and 9.0 D (water) |                           |                                     |       |
| *Brassica oleracea* seeds | CSE | CC | • T (°C): 50, 60, 70, 80, 90                                           | Total extractable components | Second order polynomial             | [15]  |
|                               |                    |     | • Power (W): 100, 125, 150, 175, 200                                   |                           |                                     |       |
|                               |                    |     | • Solvent concentration (methanol/water, v/v (%)): 50, 60, 70, 80, 90 |                           |                                     |       |
|                               |                    |     | • Time (min): 1, 7.5, 14, 20.5, 27                                     |                           |                                     |       |
| Biomass                              | Extraction process | DOE | Factors                                                                 | Response | Model                          | Ref |
|-------------------------------------|--------------------|-----|-------------------------------------------------------------------------|----------|--------------------------------|-----|
| Red Cabbage (Brassica oleracea)     | UAE                | BB  | • Ultrasonic time (min): 20, 40, 60<br>• Ultrasonic frequency (kHz): 0, 22.5, 45<br>• T (°C): 50, 60, 70 | TPC      | Second order polynomial        | [16]|
| Broccoli leaves                     | CSE                | CC  | • Pressure (bar): 150, 225, 300<br>• T (°C): 35, 47.5, 60<br>• E (%): 0, 10, 20<br>• Time (min): 10, 20, 30 | AA       | Second order polynomial        | [17]|
| Rapeseed stems (Cultiva Dicetorm)   | UAE                | BB  | • Ultrasound power (W): 0, 200, 400<br>• Treatment time (min): 5, 32.5, 60<br>• Sample length (cm): 0.5, 1.5, 2.5<br>• Agitation speed (rpm): 0, 300, 600 | TPC      | Second order polynomial        | [18]|
| White cabbage Brassica oleracea var. capitata | UAE                | CC  | • T (°C): 30, 36, 50, 64, 70<br>• E (%): 20, 32, 60, 88, 100 | Phenolic compound content (HPLC) | -                              | [19]|
| Mustard seed (Sinapis Alba L.)      | UAE                | CC  | • Solvent polarity: 32.6, 56.3, 78.4<br>• Ultrasound power to sonication time ratio (W/min): 0.4, 8 | TPC, AA  | Second order polynomial        | [20]|
| Canola seed cake (Brassica napus)   | MAE                | BB  | • Time (min): 1, 3, 5<br>• Microwave power (W): 440, 770, 1100<br>• S/M (mL/g): 4, 5, 6 | TPC, TFC, AA | Second order polynomial        | [21]|
| Broccoli (Brassica oleracea)        | Alkaline Hydrolysis and UAE | BB  | • T (°C): 40, 60, 80<br>• NaOH concentrations (M): 1, 2, 4<br>• Sonication time (min): 15, 30, 45 | -        | Second order polynomial        | [22]|
| Cauliflower Waste (Brassica oleracea) | UAE               | 3³ factorial | • Solvent volume (mL): 50, 75, 100<br>• Extraction time (min): 20, 30, 40<br>• T (°C): 50, 60, 70 | TPC      | First-order polynomial         | [23]|
| Biomass                                      | Extraction process | DOE | Factors                                                                 | Response | Model                          | Ref |
|---------------------------------------------|--------------------|-----|-------------------------------------------------------------------------|----------|--------------------------------|-----|
| Canola seed cake (*Brassica napus*)         | CSE                | Orthogonal experiment | • T (°C): 30, 50, 70<br>• Liquid-to-solid ratio (mL/g): 10, 15, 20<br>• Time (min): 30, 60, 90<br>• E (%): 45, 70, 95 | AA       | First order polynomial        | [24] |
| Maca flour (*Lepidium meyenii*)             | CSE                | BB  | • T (°C): 30, 50, 70<br>• Liquid-to-solid ratio (mL/g): 10, 15, 20<br>• Time (min): 30, 60, 90<br>• E (%): 45, 70, 95 | TPC, AA  | Second order polynomial       | [25] |
| Broccoli                                   | MAE                | CC  | • T (°C): 50, 60, 70, 80, 90<br>• Power (W): 100, 125, 150, 175, 200<br>• Solvent concentration (methanol/water, v/v (%)): 50, 60, 70, 80, 90<br>• Time (min): 1, 7.5, 14, 20.5, 27 | -        | -                             | [26] |
| Purple Cabbage                             | MAE                | CC  | • Power (W): 100, 200, 300, 400, 500<br>• Liquid to solid ratio: 35, 47.5, 60<br>• E (%): 40, 50, 60, 70, 80<br>• Time (min): 25, 40, 55, 70, 85 | Yield of proanthocyanidins | Second order polynomial | [27] |
| Fresh cabbage *Brassica oleracea var. capita* | CSE                | CC  | • S/M (mL/g): 10, 15, 20, 25, 30<br>• E (%): 0, 25, 50, 75, 100<br>• T (°C): 35, 50, 65, 80, 95 | TPC, AA  | Second order polynomial       | [28] |

Table 1. Studies dealing with the extraction of PCs from Brassica.
3. Optimization of sinapine extraction from mustard bran

3.1 Context of the study

Mustard bran is one of the main by-products of the mustard seed industry whose production peaked at 710 thousand tonnes in 2018 [29]. By-products from their processing represent up to 60%w of seeds [30]. Mustard bran is rich in water with a content between 53 ± 1%. The dry matter is mainly composed of proteins (27 ± 1%), lipids (18 ± 1%), carbohydrates (34 ± 5%) and ash (12 ± 5%) [30–32].

Phenolic compounds represent between 1 and 4% of the wet matter of defatted mustard seeds [33]. They are mainly derivatives of sinapic acid, present at 90% as sinapine with relatively small amounts of sinapic acid. Sinapine can be used directly due to its many bioactivities [5, 34] or be hydrolyzed to sinapic acid by chemical or enzymatic means [35]. Thus, our work will focus on the extraction of sinapine from mustard bran. Moreover, bio-based sinapic acid is highly sought after thanks to its many applications, whether in cosmetics (anti-aging, anti-UV) or in the field of polymers [6, 8].

Thus, the implementation of a green extraction process to recover sinapine seems particularly relevant. The most widely used process in the various studies found in the literature is conventional solvent extraction (CSE). This is a solid/liquid extraction, the liquid being a solvent whose properties will define the sustainability of the process. Solvents such as acetone, methanol, ethanol or water, as well as a mixture, have been used [36]. To follow the principles of green extraction [37], the extraction process developed will use aqueous ethanol as solvent, the percentage of which will be determined during the optimization of the process.

3.2 Material and methods

Mustard bran, was supplied by Charbonneaux-Brabant (Reims, France). Mustard (B. juncea) grew in Canada and was processed in France. The treatment undergone by the seeds is cold mechanical pressure. The material has not been defatted, ground or dried. The raw mustard bran was stored in a cold room at 4°C until use.

A CSE using an ethanol/water mixture was implemented to remove sinapine from mustard bran. A fixed volume of 100 mL of solvent was used for each experiment. The extraction temperature was regulated with a digital thermometer in contact with the solvent and connected to the heating plate (IKA-RCT). Magnetic stirring was ensured throughout the duration of the extraction (2 h). Centrifugation was used (4713 g, 10 min) to separate the liquid extract from the solid residue. The sinapine content was measured by HPLC. More details on the materials and the methods can be found in Reugoat et al., 2020 [1].

3.3 Implementation of RSM

RSM is the recommended approach to optimize process operating conditions, for example to maximize extraction yield or minimize impurity content. Indeed, the implementation of the RSM, and therefore of a design of experiments, makes it possible to minimize the number of experiments, to determine the quadratic effect of a factor or the interaction between several factors and to obtain a high precision on the prediction of an optimal value.

The implementation of RSM requires the identification of the factors that will be involved in the model. Thus, RSM is often used after a screening plan which allows
the discrimination of the operating conditions leading to a significant variation in
the response. Sometimes, prior knowledge of the process is sufficient to avoid the
screening step and RSM can be applied after arbitrary choice of factors by the
experimenter. RSM is a relevant approach if the response surface is suspected to be
curved. Indeed, the equation of the model used includes quadratic terms which
make it possible to translate the curvature of the response.

In order to apply RSM, it is necessary to follow a rigorous approach so as not to
end up with wrong conclusions or an unusable data set for the prediction of an
optimum. This approach is illustrated in Figure 1.

For each step, the reasoning adopted for our case study will be detailed, the
choices will be explained so that the methodology can be easily implemented on
other cases.

3.3.1 Definition of the objectives

The objective of the optimization study must be defined according to the overall
objective of the application. In our case, the operating conditions of the extraction
process leading to a maximum yield of sinapine are sought. However, the global
objective of the application is to produce sinapine, that is, to obtain a high purity
sinapine extract. Thus, a second variable to be optimized emerges in addition to the
yield of sinapine: the purity of the sinapine extract. Under such considerations, the
optimum operating conditions sought will be a compromise between those allowing

![Methodology for the implementation of RSM](image)
to maximize the yield of sinapine and the ones that maximize the purity of sinapine. Failure to correctly define the objective may lead to an incorrect definition of the responses, factors and their levels and thus induce a partial conclusion at the end of the study.

3.3.2 Definition of the responses

A response is defined as a variable to be explained. For the choice of responses, it is necessary to ensure that the measurement tools are sufficiently repeatable. Indeed, in statistics, it is common to say that the more the value is dispersed the more it will be difficult to highlight significant differences and therefore to obtain a valid prediction model. This is why the presence of a triplicate in the DOE is essential to quantify the repeatability of the measurement. If it is too large, the DOE will not be able to generate a valid model.

In our case study, the two responses to be optimized are the yield of sinapine in % ($Y_1$) and the purity of sinapine in % ($Y_2$) defined by Eq. (1,2)

$$Y_1 = \frac{C_{\text{sinapine}} \times V_{\text{solvent}}}{m_{\text{BDM}}}$$

(1)

with $C_{\text{sinapine}}$ the sinapine content measured by HPLC in mg/L, $V_{\text{solvent}}$ the volume of solvent added during the extraction and $m_{\text{BDM}}$ the mass of dry matter in mustard bran.

$$Y_2 = \frac{m_{\text{sinapine}}}{m_{\text{EDM}}} \times 100$$

(2)

with $m_{\text{sinapine}}$ (g) the mass of sinapine in the extract determined from $C_{\text{sinapine}}$ and $m_{\text{EDM}}$ the mass of the dry matter in the extract (g).

3.3.3 Definition of the factors

A factor is defined as a variable that provides information to explain a response. Two strategies can be used to define the factors: to apply a screening plan (factorial or Plackett-Burman) or to use expertise on the process. In our case, the factors were chosen based on prior knowledge about the extraction process [4]. Note that the factors must be independent for the implementation of the experimental design. This should be checked before establishing the matrix of experiments.

According to theory, the liquid/solid extraction processes are influenced by a set of parameters which can modify their efficiency. These relate to: (i) the equipment used (stirring power, the configuration of the reactor), (ii) the operating conditions (extraction time, extraction temperature and pressure), and (iii) the biomass and the solvent (solvent-to-matter ratio, state of the biomass, nature of the solvent).

Some of these parameters are often fixed in the design of the experiments. Indeed, for laboratory experiments, the extraction reactor is always the same as well as the stirring system (type and power). Conventionally, the extraction time corresponds to the time required to reach the equilibrium. In our case, the biomass is wet and in the form of bran, so it cannot be crushed or sieved. This parameter cannot be taken as a factor. In addition, two constraints were imposed: to conduct the experiments at atmospheric pressure and working with ethanol (pure or aqueous) to design a sustainable process. Thus, the parameters that could be included as factors in the design of experiments are the solvent-to-matter ratio, the extraction temperature and the ethanol concentration. These parameters being independent, three
factors will be used in models developed using RSM. The last point to be defined is the variation range of each factor.

3.3.3.1 Range of extraction temperature

Technological limits exist for the choice of extraction temperatures. The experimental domain cannot be extended above 75°C to avoid evaporation phenomena due to the boiling temperature of ethanol. Thus, the extraction temperature will be able to vary between room temperature and 75°C. However, according to the literature, it does not seem interesting to carry out experiments at temperatures close to room temperature. Indeed, it is known that an increase of temperature allows to improve the extraction of phenolic compounds. A range of values too large can adversely affect the quality and accuracy of the prediction model. We have, therefore, chosen to limit our temperature range between 45°C and 75°C.

3.3.3.2 Range of solvent-to-matter ratio (S/M)

There is also a technological limit for this factor. Indeed, it is not possible to extract with less than 10 mL per gram of mustard bran. In addition, the objective being not to consume too much solvent, no more than 30 mL per gram of mustard bran will be used. Thus, the range of the S/M factor will be between 10 and 30 mL/g.

3.3.3.3 Range of ethanol concentration

No technological limit was found for this factor. The use of extreme values (water or pure ethanol) is not interesting because the better extraction yields are obtained with aqueous ethanol. According to preliminary experiments, to maximize the yield of sinapine (Y1), the values to be studied should be between 40 and 80%. Considering the purity of sinapine (Y2), the values to be studied should be between 60 and 100% in order to limit the extraction of impurities such as sugars and proteins. To define the range of variation of the ethanol concentration, we merged the two previous intervals by removing the extreme values so as not to widen the range of values to be studied too much. Thus, ethanol concentrations between 45 and 95% were studied in the design of experiments.

3.3.4 Choice and implementation of the design of experiments

The two most used design of experiments for the implementation of RSM are the composite center (CC) and Box–Behnken (BB) designs.

For a same number of factors and levels, a BB design generates fewer experiments than a CC design. However, BB designs have a certain rigidity in their implementation since the number of levels per factor is fixed. In addition, the BB designs do not include in the experiments the extreme values of the variation ranges of the factors. This can sometimes constitute a problem, when a precise knowledge of the interval is available and/or when the extreme values want to be tested.

In addition, CC design is to be able to integrate preliminary experiments. Thus, the results of certain experiments present in the screening plan carried out upstream can be used as experiments in the CC design. Thus, the number of new assays to realize will decrease.

The CCF design belongs to the category of the CC design. The experiments defined are located in the center of each face of the experimental domain.

In our application, a CCF design was used to optimize the extraction process for the recovery of sinapine. A total of 17 experiments including a repetition at the
central point constitutes the set of the experiments to implement RSM. The different assays are presented Table 2 in the form of coded and uncoded variables.

| Assay | $X_1$ | $X_2$ | $X_3$ | $T(°C)$ | $E$ (v/v) | S/M (mL/g_BDM) |
|-------|-------|-------|-------|---------|-----------|----------------|
| 1     | -1    | -1    | -1    | 45      | 45        | 10             |
| 2     | +1    | -1    | -1    | 75      | 45        | 10             |
| 3     | -1    | +1    | -1    | 45      | 95        | 10             |
| 4     | +1    | +1    | -1    | 75      | 95        | 10             |
| 5     | -1    | -1    | +1    | 45      | 45        | 30             |
| 6     | +1    | -1    | +1    | 75      | 95        | 30             |
| 7     | -1    | +1    | +1    | 45      | 95        | 30             |
| 8     | +1    | +1    | +1    | 75      | 95        | 30             |
| 9     | -1    | 0     | 0     | 45      | 70        | 20             |
| 10    | +1    | 0     | 0     | 75      | 70        | 20             |
| 11    | 0     | -1    | 0     | 60      | 45        | 20             |
| 12    | 0     | +1    | 0     | 60      | 95        | 20             |
| 13    | 0     | 0     | -1    | 60      | 70        | 10             |
| 14    | 0     | 0     | +1    | 60      | 70        | 30             |
| 15    | 0     | 0     | 0     | 60      | 70        | 20             |
| 16    | 0     | 0     | 0     | 60      | 70        | 20             |
| 17    | 0     | 0     | 0     | 60      | 70        | 20             |

Table 2. CCF experimental design.

$X_1$ (extraction temperature; 45, 60, 75°C), $X_2$ (concentration of ethanol; 45–70–95%v/v) and $X_3$ (solvent-to-matter ratio; 10, 20, 30 mL/g_BDM) are the independent variables used to explain the responses $Y_1$ (sinapine yield on the mustard bran dry matter in g/g_BDM) and $Y_2$ (sinapine purity on the extract dry matter in %EDM).

The experimental data were fitted using a second-order polynomial (Eq. (3)):

$$Y_q = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i<j=1}^{3} \beta_{ij} X_i X_j + \epsilon_q$$ (3)

where $Y_q$ are the different responses ($q = 1–2$); $\beta_0$, $\beta_i$, $\beta_{ij}$, $\beta_{ii}$ are the regression coefficients for the mean, linear, interaction and quadratic terms respectively. $X_i$ and $X_j$ are the independent variables, $\epsilon_q$ the residues between the observed and the predicted values.

3.3.5 Run the experiments

This step corresponds to data collection. Assays can be performed in random order. The material and methods of analysis were briefly introduced. More details can be found in Reungoat et al. (2020) [1].

3.3.6 Development and validation of the model

Once the data has been collected, they are processed by a software to generate a model and indicators that allow its quality to be assessed (fit to the data, ability to
predict). The software used to carry out our case study is the commercial software MODDE v.12.0 (Umetrics AB, Sweden).

First, it is necessary to determine whether the model should be reduced. Reducing a model means removing variables whose coefficients are not significant. Significance tests are carried out for this purpose. The p-values obtained indicate whether the value of the coefficient can be considered equal to 0. In this case, the factors are considered to have no effect on the response. Table 3 presents the scaled and centered coefficients of the model associated with each term as well as the results of the significance test for each coefficient.

The p-values in red in Table 3 indicate the significant coefficients and factors to keep in the model.

3.3.6.1 Analysis of the prediction model of $Y_1$

The significant coefficients are $\beta_0$ (constant), $\beta_1$ (T) and $\beta_{22}$ (E*E). Since the quadratic term $E^*E$ is significant, the variable $E$ cannot be removed from the model. Thus, the factors to keep are temperature and ethanol. The $S/M$ ratio has no effect on the sinapine yield. The data must be reprocessed by the software keeping the variables T, $E$ and $E^*E$. New values are found for the coefficients of the reduced model. Sinapine yield can be predicted according to Eq. (4) with unscaled coefficients.

$$Y_1 = -4.846 + 0.002 T + 0.376 E - 0.003 (E \times E) + \varepsilon$$  \hspace{1cm} (4)

3.3.6.2 Analysis of the prediction model of $Y_2$

The significant coefficients are $\beta_0$ (constant), $\beta_1$ (T), $\beta_2$ (E), $\beta_3$ (S/M), $\beta_{23}$ (E*S/M) and $\beta_{22}$ (E*E). Thus, all the factors should be kept. The data must be processed by the software by keeping the variables T, $E$, $S/M$, $E*S/M$ and $E*E$. New values are found for the coefficients of the reduced model. Sinapine purity can be predicted according to Eq. (5) with unscaled coefficients.

$$Y_2 = -6.452 + 0.010 T + 0.174 E + 0.297 (S/M) - 0.001 (E \times E) - 0.003(S/M \times S/M) - 0.003(E \times S/M) + \varepsilon$$  \hspace{1cm} (5)

| Variables | Coefficients scaled and centered | $Y_1$ | p-value | $Y_2$ | p-value |
|-----------|----------------------------------|-------|---------|-------|---------|
| constant  | $\beta_0$                        | 8.398 | < 0.001 | 3.491 | < 0.001 |
| T         | $\beta_1$                        | 0.622 | < 0.01  | 0.140 | 0.030   |
| E         | $\beta_2$                        | -0.038| 0.755   | 0.353 | < 0.01  |
| S/M       | $\beta_3$                        | -0.021| 0.863   | -0.231| < 0.01  |
| T*E       | $\beta_{12}$                     | 0.098 | 0.382   | 0.010 | 0.817   |
| T*S/M     | $\beta_{13}$                     | 0.227 | 0.070   | -0.022| 0.584   |
| E*S/M     | $\beta_{23}$                     | -0.037| 0.745   | -0.417| < 0.001 |
| T*T       | $\beta_{11}$                     | -0.136| 0.472   | -0.061| 0.473   |
| E*E       | $\beta_{22}$                     | -0.745| < 0.01  | -0.388| 0.007   |
| S/M*S/M   | $\beta_{33}$                     | -0.258| 0.194   | -0.121| 0.185   |

Table 3. Values of the model coefficients and the p-values of their significance tests.
Secondly, the indicators calculated on each reduced model are interpreted to assess whether the correlation between the model and the experimental data is acceptable and whether these models can be considered as good prediction tools. These indicators are presented in Table 4.

| Indicators                          | Reduced model (Y₁) | Reduced model (Y₂) |
|-------------------------------------|--------------------|--------------------|
| $R^2$                               | 0.90               | 0.97               |
| $R^2_{adj}$                         | 0.86               | 0.94               |
| Model regression (p value)          | 0.00003            | 0.00002            |
| Reproducibility                     | 0.98               | 0.99               |
| Condition number                    | 2.88               | 4.67               |

Table 4. Indicators to assess the fit and quality of reduced models.

The coefficients of determination being close to 1, the reduced models have a good accuracy in their prediction. The values of the adjusted coefficients of determination are high enough to suggest a satisfactory correlation between the values predicted by the model and the values observed by the experiments. The p-values obtained by the ANOVA on the model regression are less than 0.01% which validates the models obtained. The condition number determines the correct orthogonality of the two models because it does not exceed 10. Each model reproducibility is also excellent with a value close to 1. All these statistical parameters indicate that the relationships between the variables and the responses are well described by the models.

3.3.7 Determination of the optima and validation of the model

In order to determine the optimal operating conditions for each response, the 3D response surfaces will be plotted. In a second time, the software optimizer tool based on the Nelder–Mead simplex method was implemented to obtain the optimal operating conditions. Figure 2 presents the evolution of $Y_1$ according to the extraction temperature, the ethanol concentration for a solid-to-matter ratio of 10 mL/g.

Figure 2. 3D response surface for a solid-to-matter ratio of 10 mL/g for $Y_1$. 

Response Surface Methodology in Engineering Science
Variations of the sinapine yield from 5.3 to 8.9 mg/g BDM were found among the 17 experiments of the CCF design.

As can be seen on Figure 2, the sinapine yield evolves in a parabolic shape. This can be explained by a strong influence of the quadratic term of the ethanol concentration. The maximum sinapine yield is achieved in the range 65–80% ethanol. The extraction temperature has a positive effect on the sinapine yield as observed in Figure 2 with the inclination of the response surface towards the high temperature zone.

The optimal operating conditions determined for $Y_1$ by the software MODDE are 70% ethanol, 75°C.

An experimental sinapine yield of $8.8 \pm 0.1$ mg/g was achieved under these conditions.

Figure 3 presents the evolution of $Y_2$ according to the extraction temperature, the ethanol concentration for a solid-to-matter ratio of 10 mL/g BDM.

Variations of the sinapine purity from 1.4% EDM and 4.4% EDM were found among the 17 experiments of the CCF design.

For a ratio of 20, the response surface is flat. Quadratic terms have little influence. The extract, containing the most sinapine compared to other extracted solutes, is obtained for a maximum temperature and ethanol concentration. This may be due to low solubility of proteins, sugars and minerals in ethanol compared to sinapine. However, an increase of the solvent-to-matter ratio increases the solubility of those impurities and decreases the sinapine purity in the extract.

The optimal operating conditions determined for $Y_2$ by the software MODDE are 100% ethanol, 75°C and, 10 mL/g BDM. An experimental sinapine purity of $4.4 \pm 0.1%_{\text{EDM}}$ was achieved under these conditions.

Since the two optima are not the same, it will be necessary to find the operating conditions allowing to maximize the two responses at the same time. Figure 4 presents the response surfaces for $Y_1$ and $Y_2$ on the same graph.

The MODDE software has determined that the optimal operating conditions that will provide the highest yield of sinapine while maintaining high purity, are 83% ethanol, 75°C, and 10 mL/g BDM. An experimental sinapine yield of $8.0 \pm 0.1$ mg/g was obtained under these conditions with a purity of $4.2 \pm 0.1%_{\text{EDM}}$. 
The last step to be carried out is the validation of the models on new experiments. For this, experiments were realized in duplicate under optimal conditions corresponding to the maximization of $Y_1$ and for the compromise between $Y_1$ and $Y_2$. Student’s tests were performed to determine if the predicted values given by the models can be considered equivalent to the observed values. The results are shown in Table 5.

Experimental values correspond to predicted values since p-value $> 0.05$. Thus, models developed by RSM are validated and can be used as prediction tool.

4. Conclusions

Concerning the extraction of sinapine from mustard bran, a CCF design was used to optimize the extraction process. A total of 17 experiments including a repetition at the central point constituted the set of the experiments to implement the RSM.

Two prediction models have thus been developed. These models have been validated, making it possible to predict the yield and the purity of sinapine from the...
operating conditions of the extraction process (extraction temperature, ethanol concentration and solvent-to-matter ratio).

An optimal sinapine content of $8.8 \pm 0.1\, \text{mg/g}$ was obtained at $75^\circ\text{C}$, 70% ethanol and 10 mL/g$_{\text{BDM}}$ whereas an optimal purity of sinapine in the extract ($4.2 \pm 0.1\%_{\text{EDM}}$) was achieved under different operating conditions ($75^\circ\text{C}$, 100% ethanol and 10 mL/g$_{\text{BDM}}$).

Wishing to situate us as close as possible to the 2 optima, the MODDE software determined that the most appropriate operating conditions were $75^\circ\text{C}$, 83% ethanol and 10 mL/g$_{\text{BDM}}$. The loss in yield and purity remains low since the sinapine yield of $8.0 \pm 0.1\, \text{mg/g}$ and a purity of $4.0 \pm 0.1\%_{\text{EDM}}$ are obtained.

The use of rigorous mathematical tools for optimization in process engineering remains under-exploited as we have shown for the extraction of phenolic compounds from Brassica. To remedy this, a generalization of the learning and use of experimental designs in universities and in the research community should be put in place. This is to encourage experimenters to optimize their process in a structured way rather than using OFAT approaches which seem easy to understand at first glance, but which may prevent the full exploitation of the information provided by the experiments. The case study, presented here, illustrated the potential in terms of process optimization using RSM.

Acknowledgements

The case study presented was supported by Extractis (Amiens, France). We would like to thank the Region Grand Est, the Conseil Départemental de la Marne and the Grand Reims for their financial support, as well as Charbonneaux-Brabant for providing the mustard bran.

Conflict of interest

There is no conflict of interest.

Nomenclature

| Acronym | Definition |
|---------|------------|
| AA | Antioxidant activity |
| ASE | Accelerated solvent extraction |
| BB | Box–Behnken |
| CC | Central Composite |
| CCF | Central Composite Face Centered |
| CSE | Conventional Solvent Extraction |
| E | Ethanol concentration |
| MAE | Microwave-accelerated extraction |
| PCs | Phenolic compounds |
| PEF | Pulsed electric field |
| S/M | Solvent to Matter ratio |
| T | Temperature |
| TAA | Total Antioxidant Activity |
| TPA | Total of Phenolic Acids |
| TPC | Total Phenol Content |
| UAE | Ultrasound-accelerated extraction |
References

[1] Sharma P., Gaur V.K., Sirohi R., Varjani S., Kim S.H., Wong J.W.C. Sustainable processing of food waste for production of bio-based products for circular bioeconomy. Bioresource Technology. 2021; 325. DOI: 10.1016/j.biortech.2021.124684

[2] Lizárraga-Velázquez CE, Leyva-López N, Hernández C, Gutiérrez-Grijalva EP, Salazar-Leyva JA, Osuna-Ruíz I, Martínez-Montaño E, Arrizón J, Guerrero A, Benitez-Hernández A, Ávalos-Soriano A. Antioxidant Molecules from Plant Waste: Extraction Techniques and Biological Properties. Processes. 2020; 8(12):1566. https://doi.org/10.3390/pr8121566

[3] Laguna O., Guyot S., Yu X., Broudiscou L.P., Chapoutot P., Solé-Jamault V., Anton M., Quinsac A., Sicaire A.G., Fine F., Citeau M., Durand E., Barakat A., Villeneuve P., Lecomte J., Dauguet S. The PHENOLEO project or how to separate and add-value to phenolic compounds present in rapeseed and sunflower meals. OCL-Oilseeds and fats crops and lipids, 2020; 27(61). https://doi.org/10.1051/ocl/2020056

[4] Reungoat V, Gaudin M, Flourat AL, Isidore E, Mouterde LMM, Allais F, Ducatel H, Joannou I. Optimization of an Ethanol/Water-Based Sinapine Extraction from Mustard Bran Using Response Surface Methodology. Food Bioproducts and Processing. 2020;122: 322–331. DOI:10.1016/j.fbp.2020.06.001

[5] Niciforovic N., Abramovic H. Sinapic acid and its derivatives: natural sources and bioactivity. Comprehensive reviews in food science and food safety. 2014;13 (1), 34–51. DOI: 10.1111/1541-4337.12041

[6] Baker L.A., Horbury M.D., Greenough S.E., Allais F., Walsh P.S., Habershon S., Stavros V.G. Ultrafast photoprotecting sunscreens in natural plants. Journal of Physical Chemical Letters, 2016;7, 56–61, http://dx.doi.org/10.1021/acs.jpcllett.5b02474

[7] Dean J.C., Kusaka R., Walsh P.S., Allais F., Zwier T.S. Plant Sunscreens in the UV-B: ultraviolet spectroscopy of jet-cooled sinapoyl malate, sinapic acid, and sinapate ester derivatives. Journal of American Chemical Society, 2014;136, 14780–14795. http://dx.doi.org/10.1021/ja5059026.

[8] Janvier M., Hollande L., Jaufurally A.S., Pernes M., Ménard R., Grimaldi M., Beauprand J., Balague P., Ducrot P.-H., Allais F. Syringaresinol: a renewable and safer alternative to bisphenol A for epoxy-amine resins. ChemSusChem, 2017;10, 738–746. http://dx.doi.org/10.1002/cssc.201601595.

[9] Jaufurally A.S., Teixeira A.R.S., Hollande L., Allais F., Ducrot P.-H. Optimization of the laccase-catalyzed synthesis of (±)-syringaresinol and study of its thermal and antiradical activities. 2016, ChemistrySelect;1, 5165–5171. http://dx.doi.org/10.1002/slct.201600543.

[10] Zardo I, Rodrigues NP, Sarkis JR, Marczak LD. Extraction and Identification by Mass Spectrometry of Phenolic Compounds from Canola Seed Cake. Journal of Science of Food and Agricultural. 2020;100:578–586. DOI: 10.1002/jsfa.10051

[11] Boscariol Rasera G, Hilkner MH, de Alencar SM, de Castro RJS. Biologically Active Compounds from White and Black Mustard Grains: An Optimization Study for Recovery and Identification of Phenolic Antioxidants. Industrial Crops and Products. 2019;135:294–300. DOI: 10.1016/j.indcrop.2019.04.059

[12] Flourat AL, Willig G, Teixeira ARS, Allais F. Eco-friendly extraction of sinapine from residues of mustard
production. Frontiers in Sustainable Food Systems 2009;3. DOI: 10.3389/fsufs.2019.00012

[13] Nandasiri R, Eskin NAM, Thiay-Höllander U. Antioxidative Polyphenols of Canola Meal Extracted by High Pressure: Impact of Temperature and Solvents. Journal of Food Science. 2019;84: 3117–3128. DOI:10.1111/1750-3841.14799

[14] Nawaz H, Shad MA, Rauf A. Optimization of Extraction Yield and Antioxidant Properties of Brassica Oleracea Convar Capitata Var L. Leaf Extracts. Food Chemistry. 2018;242: 182–187. DOI:10.1016/j.foodchem.2017.09.041

[15] Rauf A, Nawaz H, Shad M. Effect of Solvent Polarity and Extraction Time on in Vitro Antioxidant Properties of Brassica Oleracea Convar Capitata Var L. Seeds. Pakistan Journal of Pharmaceutical Science. 2018; 31(5): 1889–1897. DOI: 10.1016/j.fodchem.2017.09.041

[16] Oroian M, Leahu A, Dutuc A, Dabija A. Optimization of Total Monomeric Anthocyanin (TMA) and Total Phenolic Content (TPC) Extractions from Red Cabbage (Brassica Oleracea Var. Capitata F. rubra): Response Surface Methodology versus Artificial Neural Network. International Journal of Fodd engineering. 2017: 13. DOI:10.1515/ijfe-2016-0036

[17] Arnáiz E, Bernal J, Martín MT, Diego JC, Bernal JL, Recio LT. Optimisation of the Supercritical Fluid Extraction of Antioxidants from Broccoli Leaves. Food Analytical Science. 2016;9:2174–2181. DOI: 10.1007/s12161-016-0399-4

[18] Yu X, Gouyo T, Grimi N, Bals O, Vorobiev E. Ultrasound enhanced aqueous extraction from rapeseed green biomass for polyphenol and protein valorization. Comptes Rendus Chimie. 2016;19:766–777. DOI:10.1016/j.crci.2016.03.007

[19] Dal Prá V, Bolsson Dolwitsch C, Oliveira Lima F, Amaro de Carvalho C, Viana C, Cicero do Nascimento P, Barcellos da Rosa M. Ultrasound-Assisted Extraction and Biological Activities of Extracts of Brassica Oleracea Var. Capitata. Food Technology and Biotechnology. 2015;53: 102–109. DOI:10.17113/fbt.53.01.15.3533

[20] Szydłowska-Czerniak A, Tułodziecka A. Application of response surface methodology to optimize ultrasound-assisted extraction of total antioxidants from Brassica napus cultivars: Ultrasound effect on antioxidants extraction from rapeseed. European Journal of Lipid Science and Technology. 2015;117: 491–502. DOI: 10.1002/ejlt.201400310

[21] Teh SS, Niven BE, Bekhit AEDA, Carne A, Birch EJ. Microwave and pulsed electric field assisted extractions of polyphenols from defatted canola seed cake. International Journal of Food Science & Technology. 2015;50:1109–1115. DOI: 10.1111/ijfs.12749

[22] Wu H, Zhu J, Yang L, Wang R, Wang C. Ultrasonic-Assisted Enzymatic Extraction of Phenolics from Broccoli (Brassica Oleracea L. Var. Italica) Inflorescences and Evaluation of Antioxidant Activity in Vitro. Food Sciences and Technology International. 2015; 21: 306–319. DOI:10.1177/1082013214536174

[23] Gonzales GB, Smagghe G, Raes K, Van Camp J. Combined Alkaline Hydrolysis and Ultrasound-Assisted Extraction for the Release of Nonextractable Phenolics from Cauliflower (Brassica oleracea var. botrytis) Waste. Journal of Agricultural and Food Chemistry. 2014;62:3371–3376. DOI:10.1021/jf500835q

[24] Teh SS, Birch EJ. Effect of ultrasonic treatment on the polyphenol content and antioxidant capacity of extract from defatted hemp, flax and canola seed
cakes. Ultrasonics Sonochemistry. 2014; 21:346–353. DOI: 10.1016/j.ultsonch.2013.08.002

[25] Campos D, Chirinos R, Barreto O, Noratto G, Pedreschi R. Optimized Methodology for the Simultaneous Extraction of Glucosinolates, Phenolic Compounds and Antioxidant Capacity from Maca (Lepidium Meyenii). Industrial Crops and Products. 2013;49:747–754. DOI:10.1016/j.indcrop.2013.06.021

[26] Okić S, Cvjetko M, Božić Đ, Fabeh S, Toth N, Vorkapić-Furac J, Redovniković IR. Optimisation of Microwave-Assisted Extraction of Phenolic Compounds from Broccoli and Its Antioxidant Activity. International Journal of Food Science and Technology. 2012;47:2613–2619. DOI: 10.1111/j.1365-2621.2012.03143.x

[27] Li SL, Chen XM, Lin J. Optimization of Microwave-Assisted Extracting of Proanthocyanidins from Purple Cabbage and Evaluation of Antioxidant Activity In Vitro Available online: https://www.scientific.net/AMR.490-495.3500 (accessed on 15 January 2021).

[28] Kim HK, Lee GD, Kwon JH, Kim KH. Monitoring on Extraction Yields and Functional Properties of Brassica Oleracea Var. Capita Extracts. Food Science Biotechnology. 2005;14:836–840. DOI: 10.1021/jf500835q

[29] FAOSTAT. 2020. Production/Yield Quantities of Mustard Seed [WWW Document], http://www.fao.org/faostat/en/#data/QV/visualize (accessed 05.06.19).

[30] Sehwag S, Das M,. A brief overview: present status on utilization of mustard oil and cake. Indian Journal of Traditional Knowledge. 2015;14:244–250.

[31] Sarwar G, Bell JM, Sharby TF, Jones JD. Nutritional evaluation of meals and meal fractions derived from rape and mustard seed. Canadian Journal of Animal Science. 1981;61: 719–733. DOI: 10.4141/cjas81-087.

[32] Newkirk R, Classen H, Tyler R. Nutritional evaluation of low glucosinolate mustard meals (Brassica juncea) in broiler diets. Poultry Science 1997;76: 1272–1277. DOI: 10.1093/ps/76.9.1272

[33] Thiym-Holländer U, Aladedunye F, Logan A, Yang H, Diehl BWK. Identification and quantification of canolol and related sinapate precursors in Indian mustard oils and Canadian mustard products: Identification of canolol, sinapine and sinapic acid in mustard. European Journal of Lipid Science and Technology. 2014;116:1664–1674. DOI: 10.1002/ejlt.201400222

[34] Li, Y.; Li, J.; Su, Q.; Liu, Y. Sinapine reduces non-alcoholic fatty liver disease in mice by modulating the composition of the gut microbiota. Food Funct. 2019, 10, 3637–3649. DOI: 10.1039/c9fo00195f

[35] Dubie J, Stancik A, Morra M, Nindo C. Antioxidant Extraction from Mustard (Brassica juncea) Seed Meal Using High-Intensity Ultrasound. Journal of Food Science. 2013;78:E542–E548. DOI:10.1111/1750-3841.12085

[36] Galanakis CM, Goulas V, Tsakona S, Manganaris GA, Gekas V. A knowledge base for the recovery of natural phenols with different solvents. International Journal of Food Properties. 2013;16:382–396. DOI:10.1080/10942912.2010.522750.

[37] Chemat F, Abert-Vian M, Fabiano-Tixier AS, Strube J, Uhlenbrock L, Gunjevic V, Cravotto G. Green extraction of natural products. Origins, current status, and future challenges. Trends in Analytical Chemistry. 2019; 118: 248–263. DOI:10.1016/j.trac.2019.05.037