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Abstract

Experimental design plays an important role in several areas of science and industry. Experimentation is an application of treatments applied to experimental units and is then part of a scientific method based on the measurement of one or more responses. It is necessary to observe the process and the operation of the system well. For this reason, in order to obtain a final result, an experimenter must plan and design experiments and analyzes the results. One of the most commonly used experimental designs for optimization is the response surface methodology (RSM). Because it allows evaluating the effects of multiple factors and their interactions on one or more response variables it is a useful method. In this section, recent studies have been compiled which aim to extraction of plant material in high yield and quality and determine optimum conditions for this extraction process.

Keywords: design of experiments, olive, phenolic content, yield, RSM, food science

1. Introduction

The response surface methodology (RSM) is a widely used mathematical and statistical method for modeling and analyzing a process in which the response of interest is affected by various variables [1] and the objective of this method is to optimize the response [2]. The parameters that affect the process are called dependent variables, while the responses are called dependent variables [3].

For example, the hardness of a meat is affected by cooking time $X_1$ and cooking temperature $X_2$. The meat hardness can be changed under any combination of treatment $X_1$ and $X_2$. Therefore, time and temperature can vary continuously. If treatments are from a continuous
range of values, response surface methodology is useful for developing, improving, and optimizing the response variable. In this case, the hardness of meat Y is the response variable, and it is a function of time and temperature of cooking. It can be expressed as the dependent variable y is a function of \( X_1 \) and \( X_2 \).

\[
Y = f(X_1) + f(X_2) + e
\]

where \( Y \) is the response (dependent variable), \( X_1 \) and \( X_2 \) are independent variables and e is the experimental error.

Response surface is a method based on surface placement. Therefore, the main goals of a RSM study are to understand the topography of the response surface including the local maximum, local, minimum and ridge lines and find the region where the most appropriate response occurs [4].

The RSM investigates an appropriate approximation relationship between input and output variables and identify the optimal operating conditions for a system under study or a region of the factor field that satisfies the operating requirements [5, 6]. Box-Behnken designs (BBD) and central composite design (CCD) are two main experimental designs used in response surface methodology [3]. Central composite rotatable design (CCRD) and face central composite design (FCCD) has also been applied to optimization studies in recent years [7–9].

The experimental data are evaluated to fit a statistical model (Linear, Quadratic, Cubic or 2FI (two factor interaction)). The coefficients of the model are represented by constant term, A, B and C (linear coefficients for independent variables), AB, AC and BC (interactive term coefficient), \( A^2 \), \( B^2 \) and \( C^2 \) (quadratic term coefficient). Correlation coefficient (\( R^2 \)), adjusted determination coefficient (Adj-\( R^2 \)) and adequate precision are used to check the model adequacies; the model is adequate when its P value < 0.05, lack of fit P value > 0.05, \( R^2 \) > 0.9 and Adeq Precision >4. Differences between means can be tested for statistical significance using analysis of variance (ANOVA) [10].

1.1. The basic and theoretical aspects of RSM

The design of experiments (DoE) is the most important aspect of RSM. The DoE aims the selection of most suitable points where the response should be well examined. The mathematical model of the process is mostly related to design of experiments. Thus, the selection of experiment design has a great effect in determining the correctness of the response surface construction. The advantages offered by the RSM can be summarized as determining the interaction between the independent variables, modeling the system mathematically, and saving time and cost by reducing the number of trials [11]. However, the most important disadvantage of the response surface method is that the experimental data are fitted to a polynomial model at the second level. It is not correct to say that all systems with curvature are compatible with a second-order polynomial model. In addition, experimental verification of the estimated values in the model should be done absolutely [3].
In early stage of DoE, screening experiments are performed. If there are many variables have little or more effect on the response, the variables which have large effects on response are identified. Therefore the aim is to determine the design variables that have large effects for further investigation [12].

2. RSM application in optimization of extraction

Using Response Surface Method in the extraction studies has been of interest to many researchers in recent years [10, 13, 14]. The steps that must be followed in order to apply this method correctly are shown in Figure 1.

Recent optimization studies using the response surface method in extraction from plant materials are summarized in Table 1. Independent and dependent variable numbers and the optimization designs are also demonstrated in the same table.

2.1. Yield

Extraction yield is one of the main properties determining efficiency of olive oil extraction. This parameter indirectly takes into account the oil content held in vegetable water and pomace [15, 16].

Extraction yield is defined as the percentage of the extracted olive oil from the total weight of fruit (g). The extraction yield is calculated using the formula below [10]:

\[
\text{Yield} = \frac{\text{Extracted Oil (g)}}{\text{Olive Fruit (g)}} \times 100
\]  

Aydar et al. used olive fruits (Olea europaea L.) from Edremit cultivar grown in Mut area were harvested in the 2015 crop season with a maturity index of 3.35 to obtain ideal conditions for an ultrasound assisted olive oil extraction. It was aimed an extraction for extra virgin olive oils in low acidity and high yield using the Box-Behnken design to optimize extraction parameters including ultrasound time, ultrasound temperature and malaxation time [10].

In terms of yield, the independent variable \((X_2)\), the quadratic term \(X_2^2\) and the interactive terms \((X_1X_2, X_2X_3)\) were all significant \((P < 0.05)\). The quadratic regression model for AV was as follows:

\[
\text{Yield} = 7.48 + 0.9062 \times X_2 + 0.8875 X_3 - 1.1X_1X_2 + 0.4375 X_2X_3 - 1.3525 X_2^2
\]

The most significant effect on the extraction yield \((P < 0.05)\) was the malaxation temperature among all ultrasound extraction variables. Conversely, ultrasound time showed no effect \((P > 0.05)\) on the yield [10].

The response surface methodology has been applied to determine the optimization of olive paste heating and how it is affected by the independent process variables including olive
paste flow (Q), high power ultrasound (HPU) intensity (W), olive temperature (OT), olive moisture (OM) and olive fat content (OF) by Bejaoui et al. [17]. They obtained a 2FI (two factor interaction) model for olive paste temperature according to the analysis of variance which showed that the regression model was significant for a P-value <0.0001. The most significant terms of the model were Q, W and the interaction terms Q*W and W*OF based on P-values less than 0.0001 [17].

Second-order equations for oleuropein yield was shown in Eq. (4) [9]

\[
Yield = 0.62767 - 0.029622X_1 - 2.60 \times 10^{-3} X_2 - 0.056494 X_3 + 4.26 \times 10^{-5} X_1 X_2 + 5.07 \times 10^{-3} X_1 X_3 \\
+ 2.48 \times 10^{-4} X_2 X_3 + 1.15 \times 10^{-4} X_{12} + 2.53 \times 10^{-6} X_{23} - 0.013423 X_{23}
\]

(4)
<table>
<thead>
<tr>
<th>Extraction</th>
<th>Extraction method</th>
<th>Process parameters</th>
<th>Design method</th>
<th>Dependent variables</th>
<th>Model</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive leaf</td>
<td>Ultrasound assisted extraction</td>
<td>Solvent concentration, the ratio of solid to solvent, extraction time</td>
<td>BBD</td>
<td>Extract yield, total polyphenol content, antioxidant activity</td>
<td>Quadratic polynomial</td>
<td>[27]</td>
</tr>
<tr>
<td>Olive waste</td>
<td>Non-conventional aqueous extraction method</td>
<td>NaOH, temperature, time, mass of the waste</td>
<td>BBD</td>
<td>Total phenolic content, relative color strength</td>
<td>Quadratic polynomial</td>
<td>[29]</td>
</tr>
<tr>
<td>Olive leaf</td>
<td>Solvent-free microwave-assisted extraction</td>
<td>Amount of sample, irradiation power, the extraction time.</td>
<td>FCCD</td>
<td>Oleuropein yield and total phenolic content</td>
<td>Quadratic polynomial</td>
<td>[9]</td>
</tr>
<tr>
<td>Olive oil</td>
<td>Ultrasound assisted extraction</td>
<td>Ultrasound time, ultrasound temperature, malaxation time</td>
<td>BBD</td>
<td>Oil yield, acidity</td>
<td>Quadratic polynomial</td>
<td>[10]</td>
</tr>
<tr>
<td>Olive oil</td>
<td>High power ultrasound assisted extraction</td>
<td>Olive paste flow, ultrasound intensity, fruit temperature before crushing, olive moisture, olive fat content</td>
<td>BBD</td>
<td>Olive paste temperature</td>
<td>2FI</td>
<td>[17]</td>
</tr>
<tr>
<td>Olive oil</td>
<td>Conventional extraction</td>
<td>Malaxation time and temperature</td>
<td>CCD</td>
<td>Acidity, peroxide value, K232, K270, Total phenolic content</td>
<td>Quadratic polynomial</td>
<td>[32]</td>
</tr>
<tr>
<td>Black Carrot</td>
<td>Ultrasound assisted extraction</td>
<td>Ultrasound energy density, temperature</td>
<td>CCD</td>
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<td>Quadratic polynomial</td>
<td>[13]</td>
</tr>
<tr>
<td>Curry leaf</td>
<td>Ultrasound assisted extraction</td>
<td>Temperature, ultrasonic power, methanol concentration</td>
<td>CCD</td>
<td>Catechin yield, myricetin yield, quercetin yield, antioxidant activity</td>
<td>Quadratic polynomial</td>
<td>[20]</td>
</tr>
<tr>
<td>Rapeseed meal</td>
<td>Ultrasound assisted solvent extraction</td>
<td>Temperature, liquid to material ratio, duration and ultrasonic power</td>
<td>BBD</td>
<td>Carotenoid yield</td>
<td>Second-order (Quadratic) polynomial</td>
<td>[31]</td>
</tr>
<tr>
<td>Gac fruit peel</td>
<td>Solvent extraction</td>
<td>Extraction time, extraction temperature, solvent ratio</td>
<td>BBD</td>
<td>Total carotenoid, Antioxidant capacity</td>
<td>Quadratic polynomial</td>
<td>[30]</td>
</tr>
<tr>
<td>Coffee silverskin</td>
<td>Ultrasound assisted extraction/Microwave assisted extraction</td>
<td>Extraction time, extraction temperature, solvent ratio</td>
<td>CCD</td>
<td>Total phenolic content, radical scavenging capacity, total caffeoylquinic acids, caffeine content</td>
<td>Quadratic polynomial</td>
<td>[21]</td>
</tr>
<tr>
<td>Extraction</td>
<td>Extraction method</td>
<td>Process parameters</td>
<td>Design method</td>
<td>Dependent variables</td>
<td>Model</td>
<td>Ref</td>
</tr>
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</tr>
<tr>
<td>Brown seaweed</td>
<td>Ultrasound assisted extraction</td>
<td>Extraction time, acid concentration, ultrasound amplitude</td>
<td>BBD</td>
<td>Total phenolic, fucose, uronic acids</td>
<td>Second-order (Quadratic) polynomial</td>
<td>[35]</td>
</tr>
<tr>
<td>Hazelnut skin</td>
<td>Ultrasound assisted extraction</td>
<td>Extraction time, temperature, ultrasound amplitude</td>
<td>CCD, BBD</td>
<td>Crude polysaccharide yield, consumed energy</td>
<td>Quadratic polynomial</td>
<td>[25]</td>
</tr>
<tr>
<td>Trapa quadrispinosa stems</td>
<td>Ultrasound assisted extraction</td>
<td>Ultrasonic time, liquid to material ratio, ultrasonic temperature</td>
<td>BBD</td>
<td>Polysaccharide yield, Ferric-Reducing Antioxidant Capacity (FRAC)</td>
<td>Quadratic polynomial</td>
<td>[26]</td>
</tr>
<tr>
<td>Sphallerocarpus gracilis roots</td>
<td>Hot water extraction, Ultrasound assisted extraction</td>
<td>Extraction temperature, Extraction time, Liquid-solid ratio, Ultrasonic power</td>
<td>BBD</td>
<td>S. gracilis yield</td>
<td>Quadratic polynomial</td>
<td>[33]</td>
</tr>
<tr>
<td>Papaya seed oil</td>
<td>Ultrasound assisted extraction</td>
<td>Time, temperature, ultrasound power, solvent to sample ratio</td>
<td>SCCD</td>
<td>Yield, antioxidant activity, p-anisidine value, peroxide value, totox value</td>
<td>Quadratic polynomial</td>
<td>[18]</td>
</tr>
<tr>
<td>Pomegranate seed oil</td>
<td>Ultrasound assisted extraction</td>
<td>Ultrasonic power, extraction temperature, extraction time, the ratio of solvent volume and seed weight</td>
<td>BBD</td>
<td>Oil yield</td>
<td>Quadratic polynomial</td>
<td>[19]</td>
</tr>
</tbody>
</table>

Table 1. Summary of recent studies published on the extraction of plant materials optimized by RSM.
Where $X_1$ is the amount of sample, $X_2$ is the Microwave (MW) irradiation power, and $X_3$ is the extraction time. The researchers found that the second power of microwave intensity was the most significant parameter, followed by the amount of sample, quadratic time, and power for oleuropein yield [9].

Response surface method has been used frequently in recent years to optimize different oil extractions other than olive oil including papaya seed oil and pomegranate seed oil [18, 19].

To optimize the ultrasound-assisted extraction conditions followed by ultrahigh performance liquid chromatography (UHPLC) to achieve high catechin, myricetin, and quercetin contents, and high antioxidant and anticancer activities in the curry leaf extracts, RSM was applied by Ghasemzadeh et al. [20]. They used the central composite experimental design (3-level, 3-factorial) to determine the optimum extraction parameters affecting the extraction yields of catechin ($Y_1$), myricetin ($Y_2$), quercetin ($Y_3$), and antioxidant activity ($Y_4$) of curry leaf extracts [20].

The extraction efficiency of UAE and MAE methods was compared to a conventional solvent extraction by Guglielmetti et al. [21]. Authors used RSM with a CCD to investigate ultrasound assisted extraction (UAE) and microwave assisted extraction (MAE) of caffeoylquinic acids and caffeine from coffee silverskin (CS) at two particle size. They found that the highest caffeine content (14.24 g kg$^{-1}$ dw) with a significant reduction of extraction time was obtained by UAE [21].

Since different extraction methods have important impacts on the polysaccharide bioactivity, yield and structure, to find the best extraction method to obtain high yield of polysaccharide is crucial. Recently several researchers used RSM for optimization of polysaccharide extraction from different plant materials [22–26]. To investigate the best response surface design for optimization of polysaccharide yield (CPS) from hazelnut skin, CCD and BBD designs were studied by Yılmaz and Tavman [25]. Optimum conditions for a maximum yield of polysaccharide extraction from *Trapa quadrispinosa* stems recently determined by Raza et al. 41 min, 31.5 mL/g and 58°C were the optimum conditions for extraction time, ratio of water to material, and extraction temperature, respectively [26].

### 2.2. Phenolic and antioxidant compound extraction from plant materials

In recent years, there has been a growing interest in finding new natural sources of food antioxidants. As a main fruit crop, olive is also valued due to its phenolic-containing leaves. Optimization of ultrasound-assisted extraction of olive leaf has been studied by extraction parameters including solid/solvent ratio, time and ethanol concentration by Şahin and Şamli [27]. In order to obtain the maximum extraction performance for an ultrasound assisted extraction, 500 mg olive leaf to 10 mL solvent ratio, 60 min of extraction time and 50% ethanol composition were found to be as optimal operating conditions [27].

Shirzad et al. also studied on optimization of olive leave extraction in order to shorten the time of extraction and decrease the consumption of energy. The conditions for obtaining maximum yield of polyphenols, total flavonoids and antioxidants were optimized using RSM The effects of ultrasonic temperature (35–65°C), ultrasonic time (5–15 min), and ethanol to water
ratio (Et:W) (25–75%) were evaluated. The highest extraction yield was found to be 51% of ethanol to water ratio at 65°C for 15 min [28].

Elksibi et al. used RSM to investigate the optimization of natural colorant non-conventional extraction technique from olive waste. They studied the combined effects of extraction conditions on total phenolic content (TPC) and relative color strength (K/S) using a three-level three-factor Box-Behnken design [29].

Second-order equation for total phenolic content from olive leaf obtained by RSM was shown in Eq. (5) by Şahin et al. [9]:

\[
TPC = -0.019369 - 0.3600 \times 10^{-3}X_1 + 0.1424 \times 10^{-3}X_2 + 0.089174 X_1 X_3 + 4.53 \times 10^{-5}X_2 X_3 - 0.012889 X_21 - 2.74 \times 10^{-4}X_22 + 2.3499 \times 10^{-3}X_23
\]

where \(X_1\) is the amount of sample, \(X_2\) is the MW irradiation power, and \(X_3\) is the extraction time [9].

Agcam et al. [13] used response surface methodology to optimize ultrasound assisted anthocyanin compounds extraction from black carrot. The independent variables were temperature and ultrasound energy density which is calculated with following Eq. (6):

\[
E = \frac{P \cdot t}{M}
\]

The optimization of five different anthocyanin compounds from black carrot was conducted using CCD design with a 16 factorial experiments, 5 replicates of the central point. They obtained quadratic polynomial equations for each anthocyanin compound which were cyanidin-3-xylosyl-glucosyl-galactoside (C3XGG), cyanidin-3-xylosyl-galactoside (C3XG), monoacylated anthocyanins cyaniding-3-xylosyl-glucosyl-galactosidesinapic acid (C3XGGS), cyanidin-3-xylosyl-glucosylgalactoside-ferulic acid (C3XGGF), and cyanidin-3-xylosyl-gluco-syl-galactoside-coumaric acid (C3XGCC) [13].

Ghasemzadeh et al. [20] found that ANOVA for predicted model of antioxidant activity was significant (F-value 17.21, P < 0.0001) with a good coefficient of determination (\(R^2 = 0.98\)). They also observed that extraction variables showed significant (\(P < 0.01\)) quadratic and linear effects on the antioxidant activity and the predicted model obtained for DPPH (\(Y_4\)) was as follows:

\[
DPPH = +79.56 - 5.70 X_1 + 1.88 X_2 + 1.29 X_3 - 1.31 X_1 X_2 + 0.24 X_1 X_3 + 0.64 X_2 X_3 - 15.29 X_1^2 - 0.57 X_2^2 - 1.14 X_3^2
\]

Where \(X_1\) is the temperature, \(X_2\) is the methanol concentration, and \(X_3\) is the ultrasonic power.

Using RSM the extraction conditions including extraction time, temperature and solvent–solid ratio were optimized for maximizing extraction yields of carotenoids and antioxidant capacity from Gac fruit peel by Chuyen et al. [30]. In that study most effective solvent was
ethyl acetate and optimal extraction conditions (time, temperature and solvent-solid ratio) were 150 min, 40.7°C and 80 mL g⁻¹, respectively [30].

Box-Behnken design (BBD) with a total number of 29 experiments were conducted for four factors (temperature, liquid to material ratio, duration and ultrasonic power) and at three levels to obtain high yield of carotenoid from rapeseed meal. Optimal ultrasound assisted extraction conditions were as follows: temperature 49.6°C, liquid to material ratio 41.4 mL/g, duration 48.5 min, ultrasonic power 252.9 W [31].

Guglielmetti et al. observed a positive correlation between an increase of temperature and total phenolic content (TPC) for conventional solvent extraction and UAE; a negative effect on TPC when using MAE above 50°C. They found that temperature was the most effective process variable on extraction processes [21].

Espínola et al. used RSM to investigate the optimum extraction condition for virgin olive oil extraction from olives at three different maturation index (MI). In olives at lowest maturity index, temperature had a positive effect on polyphenol content at low malaxation temperatures, however no significant effect was determined at higher temperatures. On the contrary, malaxation time had a slight influence at lower temperatures. In higher MI olives, variations of polyphenol content were not significantly different [32].

3. Validation of the model

In the response surface method, the model that best represents how dependent variables are affected by independent variables is determined theoretically. However, experiments should be carried out to verify the reliability of the theoretically determined models under optimum conditions. Chi-Square test and t-tests are most commonly used to determine the difference between experimental and predicted values. Another method to evaluate the validation of model is to calculate experimental error between theoretical and experimental values.

The experimental and predicted values were 8.31 and 8.42% for the acidity and the yield were 0.31 g oleic acid/100 g olive oil and 0.28 g oleic acid/100 g olive oil for predicted and experimental values, respectively. These results were in good agreement with the predicted values under the optimum working condition. Therefore, the acidity value of olive oil and yield for any combination of ultrasound time, ultrasound temperature and malaxation time could be accurately predictor by the regression models obtained by RSM [10]. In the 2005–2006 season, the estimated extraction yield, acidity and peroxide index of the 3.2 MI olive samples showed that the experimental data were consistent with the model for all three dependent variables [32].

Elksibi et al. found that experimental value of 22.54 and 1120 mg/L for the color strength parameter (K/S) and the total phenolic content, respectively. While the predicted values were 23.22 and 1134 mg/L for the color strength parameter (K/S) and the total phenolic content, respectively. They determined the results obtained at the optimal combination was in agreement with the theoretical result. Therefore, the model obtained in this research was confirmed [29].
The experimental extraction yield in the hot water extraction process was 3.79 ± 0.13% and the yield in the ultrasound extraction process was 6.04 ± 0.21% under the optimum conditions, which were in good agreement with the predicted values. These results demonstrated that the extraction models were reliable and accurate [33].

15 min, 45°C and 50% amplitude was selected as an optimal level of parameters to validate the result of desirability functions. 1.69% CPS yield and 73.00 kJ energy consumption were found and the predicted values obtained by CCD and BBD were similar to the experimental values and the points of all predicted and experimental response values were correlating. Thus the model developed was significant and reliable. Studentized test results were in agreement with experimental runs which showed that all the data points were kept within the limits [34]. Validation of the regression equation and statistical model was conducted at 49.6°C, 41.4 mL/g, 48.5 min and 240 W which were temperature, liquid to material ratio, extraction time and power of ultrasound, respectively. With these optimized conditions, the predicted response for carotenoid yield was approximately 0.1570 mg/g, and the experimental value was found as 0.1577 ± 0.0014 mg/g. These results confirmed that experimental values are in agreement with the predicted values, thus the model was validated [31].

4. Conclusions

Response surface methodology with a wide range of applications in food science and technology has been successfully used for many years. Optimization of the extraction of plant materials known to be useful for health has attracted many researchers in recent years. This section summarizes the recent researches that optimize extraction conditions necessary to obtain higher quality and yield than plant materials using RSM. One of the most important points in the implementation of this method is that the predicted values in the model should be verified experimentally. RSM has many advantages when compared to classical methods. It needs fewer experiments to study the effects of all the factors and the optimum combination of all the variables can be revealed. The interaction (the behavior of one factor may be dependent on the level of another factor) between factors can be determined. It also requires less time and effort. With all of these advantages, it will be used not only in food science but also in other areas in future.

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