Section 1
1 The use and importance of design of experiments (DOE) in process modelling in food science and technology

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ABSTRACT

In the last ten years, the use and applications of mathematical modelling have increased in chemistry and food science and technology. However, it is still common to find researchers using the ‘one at a time’ approach to test and select variables to develop and optimize products and processes. In this regard, the objectives of this review are to provide some statistical information related to mathematical modelling of processes using design of experiments followed by multiple regression analysis, the so-called response surface methodology (RSM), and to discuss some recent published researches based on RSM optimization of products and processes, with special attention to microbiology, sensory analysis, food development and nutrition.

INTRODUCTION

The development of food products and/or processes is a complex, expensive and risky multistage process, and special requirements should be considered in this process, such as consumer demands, price, operational conditions and legislation background. To develop or to optimize processes, many companies use statistical approaches, such as response surface methodology (RSM), in their research department in order to achieve the best combination of factors that will render the best characteristic of a product and or process response. In food and chemical companies, RSM has important applications in the design, analysis and optimization of existing products and unit operations, its use decreasing thus the volume of experiments, reagents, time, financial input, energy, among others (Montgomery, 2009).

Mathematical modelling for food development or unit operations to produce a food is increasing and some statistical techniques are being adopted, such as RSM, to solve problems where several independent variables (or factors) influence the response variable value (Nwabueze, 2010). In food systems, the product response of interest to the researcher might include, for example product development, functional and sensory properties, nutritional qualities, antinutritional or toxic levels, shelf life, microbiological quality, packaging performance, processing and media conditions.

It is widely accepted that RSM is a useful tool to analyse results from many different experimental responses (chemical, sensory, physicochemical etc.). Within this context, the objectives of this review are to provide some useful information regarding mathematical modelling by using design of experiments (DOE) followed by response surface methodology, and to discuss some recent published researches based...
on RSM optimization of products and processes, with special attention to microbiology, sensory analysis, development of foods products, and nutrition.

**OVERVIEW OF EXPERIMENTAL DESIGNS**

Types of design

In accordance with Montgomery (2009) and Myers et al. (2009), there are several experimental designs that can be applied in food/chemical companies to test ingredients and/or to prepare or reformulate a new food product or even to optimize the conditions to lead to an optimal process. Some of these designs are: full factorial design, fractional factorial design, saturated design; central composite design and mixture design. The use of one of these types depends on the purpose and it is important to note that, in order to achieve a final objective, sometimes it is necessary to use a sequence of two or more designs.

A full factorial design is applied when the purpose is to determine which factors (independent variables) are important in the study and the range of values (levels) of these factors. This is the only design that can evaluate interaction among all factors. Michel et al. (2011) used a two-level full factorial design to assess the effects of factors (extraction time, irradiation power, number of cycles) and their first order interactions on the extraction of antioxidants from sea buckthorn berries by using the pressurized solvent-free microwave assisted extraction technique (PSFME). The best extraction conditions were found and this method was compared to other common extraction techniques, such as pressing, maceration and pressurized liquid extraction; the authors concluded that PSFME leads to the most active and richest extract in phenolic content from buckthorn.

For two-level factorial designs ($2^k$), the mathematical model used to describe the relationship between factors and the response variable is linear:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k + \varepsilon$$  (1.1)

Thus, it is not possible to think about optimize this process. It is common for people use this type of design and find ‘optimum values’ for the factors selected. Indeed, they are obtaining the best values for the factors, considering the experimental region analysed. However, in several studies, there is an interest in determining which factor level takes the response variable to a maximum or a minimum. Therefore, a more complex model should be proposed to take into consideration the plane curvature formed by the factors and the response variable. In this case, it is possible to work with a three-level factorial design or with a central composite design; in both cases, the parabola is a mathematical model that accomplishes this objective.

In a recent study, Ellendersen et al. (2012) used a $2^2$ design to study the influence of temperature and fermentation time on the viability of *Lactobacillus casei* and *L. acidophilus* in apple juice. The best conditions to produce a probiotic apple juice were found to be 10 hours fermentation at 37°C.

Kliemann et al. (2009) evaluated the effect of four independent variables (acid, temperature, pH and extraction time) on pectin extraction from passion fruit peel using a $2^4$ factorial design, followed by a central composite design with five levels for the three statistically significant factors (temperature, pH and extraction time); the results were analysed by response surface methodology. The optimal conditions for maximum pectin yield were citric acid at 80°C and pH 1, with an extraction time of 10 minutes, when they considered a model extrapolation. The authors concluded that RSM was a suitable technique to optimize a process that makes good use of a commonly discarded product.

If it is necessary to optimize a process, the design to be used is $3^k$ or central composite design, because they allow quadratic models, as shown by Equation 1.2 for only two factors.

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \varepsilon$$  (1.2)
A three-level design ($3^k$) is not the most efficient way to model a quadratic relationship; the central composite design is preferred and requires fewer assays to achieve a better modelling. Gonzalez-Barreiro et al. (2000) tested response surface experimental design to optimize the solid phase microextraction (SPME) of the widely used herbicide alachlor. A three-level factorial design ($3^2$) was used to study the effect of extraction time and desorption time on the extraction efficiency and also to optimize the experimental conditions. The extraction time only appeared statistically significant, because the lower level for desorption time (15 min) is long enough to produce the complete desorption of the alachlor extracted by the fibre. No significant interactions were detected.

When there are many factors to be studied and there is not much time or raw materials, it is recommended to use the fractional factorial design, aiming at reducing the number of assays. Even though the accuracy of the design is lower, less time and money are spent. Zanariah et al. (2012) used a two-level half factorial design for five factors ($\text{HNO}_3$ and $\text{H}_2\text{O}_2$ volumes, sample weight, microwave power and digestion time), which involved 16 experiments, to quantify arsenic in shrimp paste samples treated by a microwave digestion method. They concluded that only two factors (sample weight and microwave power) and their interaction effects were statistically significant. The authors proposed a regression model to predict arsenic concentration, considering the main effects of sample weight and microwave power, the interaction effect between them and the interaction effect between microwave power and digestion time. Because the main effect of this last factor was not statistically important, the authors did not consider it in the mathematical model. This is a common error made by some researchers; it is necessary to consider the main effects of factors that are not statistically significant if their interaction effects with other factors are.

A very widely used $3^{k-p}$ fractional factorial design is Box–Behnken, because it considers more experimental points (allowing then more degrees of freedom, which implies a more precise analysis) than the normal fractional factorial, but less than the full factorial design. This type of design is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching optimum conditions of studied factors for desirable responses (Haaland, 1989). For example Granato et al. (2010a) used a $3^2$ design to develop a soy-based guava dessert where guava juice and soy protein were the independent variables, and the responses were the sensory properties and physico-chemical characteristics of such products. The authors obtained significant RSM models and concluded that RSM was an adequate approach for modelling the physicochemical parameters and the degree of liking of creaminess of desserts.

A $3^K$ factorial Box–Behnken design was used by Jo et al. (2008) to determine the effect of three independent variables (glucose content, pH and temperature) on the hydrogen production rate, and to optimize the process to achieve improved hydrogen production. Thus, by using RSM with the Box–Behnken design, the authors concluded that the maximum hydrogen production rate by C. tyrobutyricum JM1 ($5089 \text{ ml H}_2 (\text{g dry cell h})^{-1}$) was obtained under the optimum condition of glucose concentration $= 102.08 \text{ mM}$, temperature $= 35^\circ\text{C}$ and pH $= 6.5$.

The extreme case of fractional factorial design is the saturated design, where there are not enough degrees of freedom to calculate the interaction effects among some factors, as the number of factors (more than 11, for example) is quite high and the cost and time involved would make the use of factorial designs prohibitive. When there are many factors to be tested, the Plackett–Burman design may be an excellent option, once it has been widely used to develop process conditions and to allow the understanding of the effects of various physicochemical, biochemical and sensory variables using a minimum number of experiments. The Plackett–Burman design is widely used in food researches because it allows the screening of main factors from a large number of variables that can be retained in the further optimization process (Siala et al., 2012). For example Siala et al. (2012) used a Plackett–Burman design to analyse the effect of various conditions related to the composition of the medium, inoculum size and temperature of fermentation, totalling 11 independent variables. The authors verified that monopotassium phosphate
(KH₂PO₄), pH, and temperature were the three most significant factors; then they used a Box–Behnken design of RSM to optimize protease production by Aspergillus niger I1.

As mentioned previously, when it is necessary to optimize (to find maximum/minimum values) a response variable, it is necessary to use a 3ᵏ or central composite design (CCD), the latter being the better. But first, it is necessary to be sure that the appropriate region of the factors, where the curvature is statistically significant, has been selected. In this case, a **quadratic or second order model**, Equation 1.3 for two factors, should be applied (Nwabueze, 2010).

\[
Y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{i<j} \beta_{ij} x_i x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \epsilon
\] (1.3)

Second order models are mathematically more complex and used in biochemical reactions and sensory analysis, among others. They would likely be useful as an approximation to the true response surface in a relatively small region. The second order model is very flexible. It can take on a wide variety of functional forms, so it will often work well as an approximation to the true response surface (Keshani et al., 2010).

A central composite design was employed to optimize the extraction conditions of sapodilla juice using hot water extraction (Sin et al., 2006). The independent variables were juice extraction time (30–120 min) and temperature (30–90°C). The combined effects of these variables on juice yield, odour, taste and astringency were investigated. Results showed that the generated regression models adequately explained the data variation and significantly represented the actual relationship between the independent variables and the responses. Higher temperature increased the juice yield, taste and odour but also showed an increase in astringency, which affected the acceptability of the juice. The contour plots showed the relationships among the independent variables and the responses. All regression models were statistically significant (\(p < 0.01\)) and there was no lack of fit. A superposition of all contour plots allowed the optimum condition to be determined as 60°C for 120 minutes for hot water extraction of sapodilla juice.

The **mixture design** should be used when proposing a new formulation or a new food product. This design allows the determination of the ideal composition of each component in a mixture, with the purpose of achieving a product with the best features (taste, odour, texture, etc.). Several functional and fruit-based products have been developed using a mixture design, including desserts, smoothies, juices and pulp concentrate, among others. Pelissari et al. (2012) developed films composed of cassava starch, chitosan and glycerol by blown extrusion using a design for constrained surfaces and mixtures. The effects of the mixture components on the mechanical properties, water vapour permeability (WVP) and opacity of the films were studied. The authors concluded that the design for constrained surfaces and mixtures was a useful tool for this type of study and complexity of film formation conditions.

**Some Considerations**

According to Calado and Montgomery (2003), regardless of the design type that will be employed, some considerations should be taken into account prior to collect experimental data:

- Definition of the variables, which can be qualitative (additive type, presence of magnetic agitation, presence of light, etc.) and quantitative (ingredient concentrations, temperature, pressure, etc.).
- Definition of the relevant levels of each independent variable. This can be done by performing an initial experiment.
- Analysis of the results and of the need for relevant changes in the initial design.
A relevant issue to be addressed is the block variable. There are some variables that act as covariates because they indirectly have some influence on response variables, but they should not have. However, imagine that there are many experiments to run and it is not possible to finish all at the same day; the environmental conditions may change day by day. Thus, the day is one of these covariates. Some others are: manufacturer, operator, batch, parts, and so on. There are many examples showing that if significant block variables are not taken into account statistically, the analysis may give the wrong answer, because important factors can be wrongly considered insignificant. If the block variable is identified as not statistically important, it may be considered a replicate, increasing then the degree of freedom. For example imagine that it is necessary to measure the influence of the tip (the only real factor) of an instrument to measure the hardness of a material. There are four different parts of the same material that are supposed to have the same properties. Because it is known that they can be different, the variable ‘part’ is used as a block. After running the analysis of the experiments, it was concluded that the block was statistically significant as well as the tip. But, if the different parts had been considered replicates, it would have been concluded that the tip was not important for measuring the material hardness, which would be a wrong conclusion.

Regardless of the type of design a researcher uses, it is demanding and essential to test the statistical quality of the results prior to their evaluation. If they are not statistically good, the analysis of the design will lead to misleading conclusions. Herein, the coefficient of variation (CV = standard deviation/mean) for each dependent variable should be calculated and if the results are below 10%, they might be considered excellent, while values up to 20% are considered acceptable. For sensory that uses consumers as panelists, which is subjective by nature, the coefficient of variation may reach values as high as 40% and it can be still considered acceptable. For other applications, such as agriculture, biotechnological processes, microbiology and clinical protocols, the coefficients of variation are high because of a wide dispersion in data. In these cases, it is recommended to establish suitable and acceptable limits. For data that are homogeneous, a CV higher than 30% is considered very bad and the experiments should be repeated.

Once the mathematical model has been selected, it is important to determine its significance by means of a variance analysis (ANOVA). To do that, the standard deviations of the main and the interactions effects of the selected factors should be calculated. If the standard deviations present a lower value than the mean values, it is possible to assume that the mathematical model is significant. If this does not happen, the experimental data should be evaluated in order to not presume that the effect is not significant.

In the evaluation of experimental designs, a mathematical model is provided to relate the response variable with the factor effects. In this regard, the goodness-of-fit of this model needs an assessment and the following criteria should be analysed:

- standard deviation of the estimated parameters and model;
- statistical significance of the estimated parameters;
- regression coefficient;
- value of the objective function;
- significance of the regression (ANOVA);
- analysis of the residuals.

It is considered a good fit to the experimental data when:

- the standard deviation of the parameter presents a lower value than the correspondent effect, indicating that the standard deviation of the proposed mathematical model is low;
- the parameters of a model need to be significant, otherwise they will not contribute to the model;
- it is a myth to consider that if the model presents a regression coefficient ($R^2$) above 90%, then it is considered excellent. This is only one criterion to evaluate the model goodness-of-fit. If a regression
coefficient is low (<70%), the mathematical model is not good and, on the other hand, if its value is high (>90%), it means that other statistical criteria may be used. It is noteworthy emphasizing that depending on the type of analysis, a regression coefficient may be considered good above 70%, such as what happens in sensory evaluation data;

• the value of the objective function should be low;
• the proposed mathematical model must be statistically significant;
• the analysis of the residuals consists in verifying if these residuals (experimental value for a response variable minus predicted value by the mathematical model) have a normal distribution and if the variance is constant. This is a necessary condition for the application of some post hoc tests, such as $t$ and $F$. To test the validity of a normal distribution, quantitative tests need to be employed, such as Kolmogorov–Smirnov, Liliefors and Shapiro–Wilks. To test the variance constancy, Levene’s test is usually used.

**RESPONSE SURFACE METHODOLOGY: A TOOL FOR ANALYSING AND OPTIMIZING PRODUCTS AND PROCESSES**

Response surface methodology consists of a group of mathematical and statistical techniques used in the development of an adequate functional relationship between a response variable ($y$) and a number of associated control variables denoted by $x_1, x_2, \ldots, x_k$. In general, such a relationship is unknown but can be approximated by a low-degree polynomial model of the form

$$y = f(x)\beta + \epsilon$$

(1.4)

where $x = (x_1, x_2, \ldots, x_k)'$, $f(x)$ is a vector function of $p$ elements that consists of powers and cross-products of powers of $x_1, x_2, \ldots, x_k$ up to a certain degree denoted by $d$ ($\geq 1$), $\beta$ is a vector of $p$ unknown constant coefficients referred to as parameters and $\epsilon$ is a random experimental error assumed to have a zero mean. This is conditional on the belief that a model, which must be significant statistically, provides a suitable representation of the response and the lack of fit is not significant ($p < 0.05$) (Khuri and Mukhopadhyay, 2010).

Simple mathematical models are used to fit experimental data. Usually, linear and quadratic models are sufficient to model sensory, biochemical, physical and physicochemical data (Dutcosky et al., 2006; Capitani et al., 2009; Farris and Piergiorgio, 2009).

The first step in using surface response methodology is to determine a mathematical relationship between the response variable and the independent variables. This relationship is quantitative, covers the entire experimental range tested and includes interactions (if present). Thus, the model can be used to calculate any and all combinations of factors and their effects within the test range (Iwe et al., 2004). The response surfaces are represented mathematically by equations called models, which are similar to the well-known regression equations. First or second order regression models could be used for the analysis of responses $y$ as a function of independent variables. A brief summary of all steps that should be taken to build a response surface and then a mathematical model is presented in Figure 1.1 and Figure 1.2.

It is clear that the first model to be considered should be a straight line, as it is the simplest one. Linear behaviours usually occur in physicochemical analysis of ingredients mixture, such as pH, water activity, instrumental colour and titratable acidity. Equation 1.1 represents a first order model, as presented before.

First order models may not be able to adequately predict the response if there is a complex relationship between a dependent (response) variable and the independent (process) variables. If there is a curvature in the plane formed by a response variable and two other factors, then a polynomial with higher degree, such as a quadratic or second order model (Equation 1.3), should be applied (Nwabueze, 2010).
In accordance with Khuri and Mukhopadhyay (2010), the objectives of a mathematical model generated by RSM are:

- to determine a statistical significance of all factors whose levels are represented by $x_1, x_2, \ldots, x_k$;
- to establish a relationship between $y$ and $x_1, x_2, \ldots, x_k$ that can be used to predict response values for a given set of control variables;

Figure 1.1 Summary of the recommended statistical procedures used to analyse results from a design of experiments.

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Figure 1.2 Steps to obtain optimized food/process conditions.
to determine the optimum set of \( x_1, x_2, \ldots, x_k \) that results into a maximum (or minimum) response over a certain region of interest by means of a simultaneous optimization of the selected response variables. This gives information on the direction and magnitude of the influence of the factors and their combined effects on the product characteristics (Nwabueze, 2010).

By using an appropriate estimation method applied to the chosen model, the regression coefficients will be obtained and the estimated response can be easily calculated. Because the relation among the response and independent variables are usually not known a priori, different models should be tested in order to better fit the experimental data (Bas and Boyaci, 2007). For verification of the model adequacy, several techniques are used. Some of these techniques are residual analysis, scaling residuals, prediction of error sum of squares residuals and tests of lack of fit (Granato \textit{et al}., 2010a). The lack of fit is a measure of a model failure in representing data in the experimental domain (Montgomery, 2009). If there is a significant lack of fit, as indicated by a low probability value \( (p < 0.05) \), the response predictor is discarded. The overall predictive capability of the model is commonly explained by the regression coefficient \( (R^2) \), but this coefficient alone does not measure the model accuracy. \( R^2 \) is defined as the ratio of the explained variation to the total variation and is a measure of the degree of fit (Myers and Montgomery, 2002).

Many researchers use different critical regression coefficient values to determine whether the mathematical models can be considered good and predictive or just cannot be used for prediction purposes. Henika (1982) stated that for sensory data the regression coefficient must be above 85% to be considered satisfactory; however, Granato \textit{et al}. (2010a, 2010b) established that a value \( \geq 70\% \) was considered good for sensory, colorimetric and physicochemical results, while Joglekar and May (1987) suggested that for a good model fit, \( R^2 \) should be at least 80%. For the models that present a regression coefficient below 70%, it must be considered that there is a failure of the models to represent the data in the experimental domain (Myers and Montgomery, 2002). However, in many food science and technology applications, such as enzymology, kinetic studies and sensory evaluation, it is not surprising if no mathematical model can be adequately fitted to the experimental data. For example the affective tests, used to determine consumer acceptance of a food, is extremely subjective depending on the sensory method that is applied (Shihani \textit{et al}., 2006; Nikzadeh and Sedaghat, 2008); hence, there is a great variance among the scores given by the assessors and no mathematical model can be successfully used to model the scores.

**PROCESS OPTIMIZATION**

In accordance with Bas and Boyaci (2007), the development of a food product and the evaluation and optimization of a process are affected by numerous factors (chemical, operational, physical, sensory and physicochemical). Once it is not possible to identify the effects of all factors, it is necessary to select those ones that have major effects. Screening experiments are useful to identify the independent variables (factors) and factorial designs may be used to achieve this objective. After identifying the important factors, the improvement direction is determined and the levels of the factors are identified. Determining these levels is important because the success of an optimization process is directly related to them. Mistakenly chosen levels result in an unsuccessful optimization.

To determine the best conditions (factor levels that result in the desirable values to a response variable) to develop a product, some researchers optimize only one factor at a time, keeping constant the remaining ones. This procedure is called ‘one factor at a time’ optimization. That is, the ideal level of one factor, which provided the best (maximum/minimum) value to a response variable, is defined. Another factor is then studied, keeping constant the others. This procedure continues until all factors have been analysed.
Besides laborious, this procedure is erroneous, as it does not take into account the interactions among factors (Box and Draper, 1987). This means that the supposed ‘ideal’ level of a factor is determined based on certain levels of the others. If other levels were chosen for these other factors, the result could completely change; that is, the ‘ideal’ levels of these factors would be different, resulting in a different and maybe wrong value for the response variables (Khuri and Mukhopadhyay, 2010). Therefore, all factors must be simultaneously varied, with a minimum number of assays, according to the design methodology. The major disadvantage of this technique – one factor each time – is that it does not include interactive effects among the variables and, eventually, it does not depict the complete effects of the factors on the process (Bas and Boyaci, 2007). To overcome this problem, optimization studies using RSM can be performed to obtain optimum conditions.

**Simultaneous optimization of response variables**

The main objective of optimization is to determine the levels of independent variables that lead to the best characteristics of a particular product, such as physicochemical, colorimetric, sensory and nutritional properties, without extending excessively the experiment time with a large number of assays. These procedures can be performed using a RSM approach. One of the main objectives of RSM is to determine optimum settings of the control variables that result in a maximum (or a minimum) response over a certain region of interest. This requires having a ‘good’ fitting model that provides an adequate representation of the mean response, because such a model is used to determine the value of the optimum (Khuri and Mukhopadhyay, 2010). For food processes, and especially for food development, optimization is a way to obtain ideal conditions to achieve a desired quality (physico-chemical, chemical and sensory, for example) (Myers et al., 2009). Optimization of a product is an effective strategy of accomplishing its successful development. If a food cannot be re-engineered or modified to fulfil consumer specifications, it will not succeed in the market. Hence, optimization is required and well established in many food companies.

Simultaneous optimization techniques are used when there is more than one response variable and it is necessary to find the ‘optimum points’ of the factors that fulfil all requirements for all response variables at the same time. This is an optimization problem with restriction and nonlinear programming techniques are usually used. Most researchers use a graphical approach of superimposing the different response surfaces and finding the experimental region that gives the desired values for all response variables simultaneously. This methodology, although visually attractive, is inefficient and cannot be automated.

For first order models, the method of steepest ascent (or descent) is a viable technique for sequentially moving toward the optimum response (Myers et al., 2009).

For second order models, which are the most used for food development purposes, simultaneous optimization using the desirability function technique is the recommended tool (Granato, 2010b; Cruz et al., 2010). It is based on the idea that the ‘quality’ of a product or process has multiple quality characteristics (Reis et al., 2008). The desirability approach, proposed initially by Derringer and Suich (1980), seems very promising for optimizing simultaneous response variables, besides being easily performed (Reis et al., 2008).

The general approach consists in first converting each response variable into a desirability function $d_i$, that varies from 0 to 1 (Calado and Montgomery, 2003). That is, if it is necessary to find the factor levels that take to a maximum response variable value, it is necessary to set $d_i = 1$ for high values and $d_i = 0$ for low values of this response variable. In the case a minimum response variable value is required, it is necessary to set $d_i = 0$ for high values and $d_i = 1$ for low values of this response variable. The idea is that this desirability function acts as a penalty function that leads the algorithm to regions where the desired response variable values can be found. The factor levels that take to a maximum or a minimum of the response variable are called ‘optimum points’.
Equation 1.4 expresses the global desirability function, \( D \), defined as the geometric mean of the individual desirability functions. The algorithm should search for response variable values where \( D \) tends to 1.

\[
D = (d_1 \cdot d_2 \cdot \ldots \cdot d_m)^{1/m}
\]

(1.5)

where \( m \) is the number of response variables.

This approach is not recommended for simultaneous optimization of more than four response variables because of constraints to achieve all expected results (Reis et al., 2008). Thus, for a large number of response variables, it is necessary to select those ones that characterize the product in a more specific way and possess a relation with the main quality features (Yi et al., 2009).

Before starting the searching process for optimum values of the independent variables, the appropriate model should be determined in order to describe each response variable as a function of the factors and then to find an appropriate set of operational conditions that optimize all response variables (Calado and Montgomery, 2003).

### RSM application to foods/process development/optimization

The use of optimization procedures to improve the process conditions and to obtain a product with certain characteristics is essential to companies when a high yield, low production costs and low use of energy are desired. A search for significant effects on quality parameters is demanding and, in this context, factorial designs (to eliminate some independent variables that are not statistically significant) followed by RSM (for the remaining factors) are tools that help the researchers to estimate the influence of variables on the process, by eliminating the variables that do not seem to contribute to the final product’s quality, and also to optimize the process conditions (pressure, temperature, agitation velocity, energy input, moisture etc.) in order to obtain an improved product.

Herein, some examples of RSM applications are presented for food science and technology, microbiology, food development, sensory evaluation and nutrition.

#### Applications of RSM to food science and technology

Working with açaí powder obtained from spray drying, Tonon et al. (2008) proposed a central composite design to study the influence of inlet air temperature, feed flow rate and maltodextrin concentration on the spray drying process yield, moisture content, hygroscopicity and anthocyanin retention. By using 17 experiments, the authors concluded that inlet temperature showed a significant effect on all the response variables.

Tiwary et al. (2012) used a central composite design to study the effect of pectinase concentration, cellulase concentration, hemicellulase concentration, temperature and incubation time on the stevioside extraction from *Stevia rebaudiana* leaves. The authors tested 26 experimental conditions and optimized them by using graphical and numerical approaches. A second order quadratic equation was then fitted to the data by multiple regression procedure and authors obtained a \( R^2 = 0.9776 \) and a \( p\)-value = 0.007 for the regression, showing the significance of the DOE followed by RSM to model the experimental data.

The effects of enzyme concentration (0.16–0.84 mg/100 g guava pulp), incubation temperature (36.6–53.4°C), and incubation time (0.95–11 h) on guava juice yield were evaluated by Kaur et al. (2009). A central composite design was applied to and analysed by response surface methodology, showing that enzyme concentration was the most significant factor affecting the juice yield. A quadratic model was fitted to the results and presented no significant lack of fit \((p > 0.05)\) and a satisfactory regression coefficient \((R^2 = 85.0\%)\). Optimum juice yield was obtained with 0.70 mg/100 g guava pulp, 7.27 hours of incubation time at 43.3°C, reiterating the importance and suitability of DOE and RSM on process optimization.
Jing et al. (2011) used a multiresponse surface methodology in order to optimize the anthocyanin content, glucosinolate content and clarity when working with chitosan to remove glucosinolates from radish anthocyanin extracts. The factors were the purification conditions: pH, chitosan concentration and treatment duration. The authors used a Box–Behnken model design and optimized using a desirability function approach. A second order polynomial model was applied to adjust the three response variables. The optimal purification condition was: pH = 3.92, chitosan concentration = 1.59 g/100 ml, and treatment duration = 2.74 hours. Equation 1.4 predicts a desirability value of 0.87. The authors carried out a triplicate experiment to validate the selected purification conditions and concluded that the experimental data were in good agreement with the predicted values. Relative errors between predicted and actual values were 0.9%, 0.5% and 3.0%, respectively, for anthocyanin content, clarity and glucosinolate content, indicating that the selected processing parameters could produce radish anthocyanin extracts with high quality.

Microbiology

Recent investigations have been performed in the microbiology field to address some issues related to optimization of experimental protocols, development of new culture media and processes and also to test new ingredients to produce culture media. In the past, the optimization of media compounds by the traditional ‘one-variable-at-a-time’ strategy involving changing one independent variable was the most frequently used operation in biotechnology, but it is known that this approach is extremely time consuming, expensive and incapable of detecting the true optimum conditions, especially because of the interactions among the factors (Calado and Montgomery, 2003; Siala et al., 2012), as already discussed. Nowadays, design of experiments and RSM have been more used to optimize culture conditions and medium composition of fermentation processes, conditions of enzyme reaction and processing parameters in the production of food, drugs and enzymes by fungi, bacteria and yeasts. Ramirez et al. (2001) studied the influence of some culture conditions on the final concentration of astaxanthin (a pigment of the carotenoid family) by using a 2^5 factorial design with four central points. The five factors were: temperature, carbon concentration, nitrogen concentration, pH and inoculum. The authors used two different culture media – Yucca and YM. The statistically significant factors depended on the medium. The higher astaxanthin concentration was obtained for Yucca medium and the significant main effects were pH and carbon; the interaction effects were carbon \times temperature and inoculum \times temperature. Thus, the authors decided to apply central composite design to optimize the astaxanthin concentration by considering carbon and temperature (the main effect was not important but the interaction effects involving temperature were very important) as the only factors; the others were established at 5% of inoculum, 6.0 of pH and 0.5 g/l of nitrogen, because at these levels they obtained the highest astaxanthin concentration. The predicted optimum factor levels were carbon concentration equals to 11.25 g/l and temperature equals to 19.7°C; the maximum astaxanthin concentration was then 7823 μg/l. The adjusted R^2 equalled 0.985. The typical production conditions before optimization procedure were: temperature = 22°C, pH = 5.0, carbon concentration = 6.0 g/l, nitrogen concentration = 1.0 g/l and inoculum = 5%. Under these conditions, astaxanthin concentration was only 4200 μg/l, a value 54% less than the optimum one, showing the importance of optimizing a process.

In order to optimize the pectin hydrolysis by pectolytic enzymes produced by Aspergillus niger, Busto et al. (2007) used a central composite design, totalling 46 assays, and used enzyme concentration, substrate concentration, pH, temperature and reaction time as independent variables at five different levels (2 axial points), whereas the response variables were the reactor conversion, reducing sugar concentration, endopectolytic productivity and enzymatic depolymerization productivity. The authors used full second order polynomial models to explain the experimental data and results showed that the model did not
present lack of fit and the $R^2$ ranged from 0.96 to 0.99. The optimization procedure was performed using the graphical approach and the optimum conditions were found to be 0.03% and 0.7% of enzyme and substrate concentrations, respectively, at 46°C, 1 hour of incubation time and pH 4.8.

Singhal and Bule (2009) used a Plackett–Burman design to assess the effect of medium component on the production of ubiquinone-10 (CoQ10), a vitamin-like lipophilic component with recognized antioxidant and anticancer effects. The authors varied the concentrations of glycerol, yeast extract, calcium carbonate and magnesium sulfate (independent variables) on the production of CoQ10, in a total of 30 different combinations. Data were subjected to RSM and the experimental results were fitted into a second order regression equation, which presented a $R^2 = 0.979$ and the regression was very significant ($p < 0.0001$). The optimal concentrations for the independent variables obtained from the model were 40, 17.72, 1.57 and 0.23 g/l for glycerol, yeast extract, calcium carbonate ($\text{CaCO}_3$) and magnesium sulfate ($\text{MgSO}_4$), respectively.

The kinetic activity of cellulolytic enzymes produced by *Aspergillus niger* during the solid state fermentation of potato peels was investigated by Santos et al. (2012). For this purpose, author used a $2^{3-1}$ fractional factorial design added with four central points to evaluate the influence of temperature, water content and time on the enzymatic activity of some enzymes. Pareto charts and 3D response surface plots were built to explain the influence of the factors on the responses, and the polynomial equations seemed to be suitable to describe the results once there was not significant lack of fit, the regression was deemed statistically significant, and the $R^2$ values were above 0.87. The desirability function was used to optimize the experimental conditions to maximize the kinetic activity of xylanase and the best combination of factors was: 81.92 hours of fermentation at 28.85°C, water content of 50.72%.

**Food development**

Although it is still common to find researchers using the ‘one at a time’ approach to develop food products, this method has been put aside once it fails to optimize properties of a food product or even the best combination and levels of ingredients to enhance the desired properties, such as sensory appeal, nutritional profile and cost, among others. Nowadays, food companies have attempted to use RSM to develop food products to enhance product characteristics and to optimize industrial process to obtain a desired property. By using RSM, it is possible to check the significance of each ingredient and also the interaction of ingredients on each response, which is clearly an advantage towards the ‘one factor at a time’ approach. There are numerous publications regarding the development of new foods, beverages and ingredients, but here only a few are analysed.

A low/no added pork sausage formulation was developed by Murphy et al. (2004), where the effects of added surimi (0–40%), fat (5–30%) and water (10–35%) on the physical, textural and sensory properties were analysed by RSM. In order to accomplish the objective, the authors employed a central composite rotatable design containing five levels of each factor, totalling 15 formulations. Data were fitted with second order polynomial equations and results showed that the mathematical models were highly significant ($p < 0.05$) for protein and moisture contents, hardness, water-holding capacity and shear force. The authors did not provide any other quality parameter of the RSM models, inhibiting the full evaluation of the proposed models. Peaks in RSM three-dimensional plots and contour plots were used to extrapolate the optimum level of the three variables (surimi, fat and water). Extrapolation is not a suitable technique to optimize a food product. It would be more appropriate to use the simultaneous optimization to render a potential optimized formulation.

Wadikar et al. (2010) used a central composite design to develop ginger-based ready-to-eat appetizers. The formulation varied in relation to the content of raisins, red sugar, and ginger powder; samples were analysed in terms of sensory acceptability and total sugars. The data were subjected to multiple regression analysis and 3D surface plots were built to explain the experimental results. The
quadratic polynomial equations were significant ($p < 0.05$) and the $R^2$ and adjusted $R^2$ were 0.9232/0.7849 and 0.9898/0.9716 for the sensory score and total content of sugars, respectively, showing that such models describe the actual data well. The food product was optimized by the numerical optimization procedure in order to maximize its sensory acceptability and authors observed that the optimized formulation had a shelf-life of eight months in metalized polyester pouches and contained 6.8 g/100 g of proteins, 5 g/100 g of crude fibre and 37 mg/100 g of vitamin C. This study showed that it is possible to develop new food products with enhanced functionality by using a response surface approach.

Dutcosky et al. (2006) developed tasty cereal bars with prebiotic functional properties using three sources of fibres: inulin, oligofructose and gum acacia. The authors used a simplex-centroid design, considering these three components. The response variables were degree of liking and the attributes selected (brightness, dryness of cereals flakes, banana volatile odour, cinnamon volatile odour, banana flavour, sweetness, crunchiness, hardness, chewiness). Applying the optimization technique of Derringer–Suich, two optimal formulations were detected: 50% inulin, 50% oligofructose and 0% gum acacia and/or 8.46% inulin, 66.16% oligofructose, and 25.38% gum acacia.

**Sensory evaluation**

Deshpande et al. (2008) developed and optimized the overall acceptability of a chocolate-flavoured, peanut–soy beverage by using a three-component constrained mixture design, using peanut, soy (flour or protein isolate) and chocolate syrup as independent variables. The authors tested 28 formulations and data were subjected to multiple regression analysis; the graphical optimization technique was used to maximize the consumer acceptability of the final product. The optimal combination of factors was found to be 34.1–45.5 g/100 g peanut, 31.2–42.9 g/100 g soy flour and 22.4–24.1 g/100 g chocolate syrup.

Pepper-based appetizers, developed in the form of convenient beverage mixes, were developed by Wadikar et al. (2008). They used a central composite rotatable design without any blocking. The authors tested the effect of black-gram flour, milk powder, salt and pepper powder on the overall acceptability of test samples. The experimental data were used to fit a second order polynomial equation and results showed that the regression was significant ($p < 0.05$) and the $R^2$ and $R^2_{adj}$ were 72.76 and 59.96%, respectively, indicating the model was not so suitable to express the actual results, once it presented a low adjusted regression coefficient.

With the objective of optimizing the roasting of robusta coffee (*Coffea canephora* conillon), Mendes et al. (2001) employed a two factor central composite design (3 central points, 2 levels of axial points, totalling 11 samples) to optimize the settings for roasting time and the initial internal temperature of the roaster drum on response variables of sensory attributes (aroma, flavour and colour). The models for beverage aroma, flavour and colour presented no lack of fit ($p \geq 0.05$) and $R^2$ of 80%, 70% and 96%, respectively. The $R^2$ for the predictive model of beverage colour is quite high, although those referring to the predictive models for the acceptance of aroma and flavour are also satisfactory, considering that the response variables are hedonic sensory measurements, which often show a high variation.

**Nutrition**

Numerous studies have demonstrated that spices have considerable antioxidant properties, mostly because of the amount and variety of polyphenolic compounds present in those plant extracts. In this regard, Hossain et al. (2011) used a central composite design to investigate the effects of methanol concentration and extraction temperature on the phenolic compounds and antioxidant activity measured by the FRAP (ferric reducing antioxidant power) assay. Data were fitted into a second order polynomial equation and the authors obtained high $R^2$ values – ranging from 0.952 to 0.99 for both variables. In
addition, the lack of fit results were not significant (p > 0.05) and the regression models were highly significant (p < 0.0001), proving the suitability of RSM to analyse and model the extraction of antioxidant polyphenols from spices.

Trevisan and Areas (2012) worked with a production of corn–flaxseed snacks aiming at obtaining the maximum expansion ratio (ER), as the sensory quality and the acceptance of snack foods depend mainly on this variable and texture parameters. They analysed the effects of three independent extrusion parameters (variables), moisture content (x1), temperature (x2) and flaxseed flour content (x3) on the ER. By using a centre composite design, the authors concluded that the factor levels that resulted in a maximum expansion ratio (3.93) were: humidity = 19%, temperature = 123°C, and flaxseed content = 25%.

Martínez et al. (2004) used a second order fractional factorial design including three levels for each factor (carrot, rice, pea/potato, chicken/veal liver) to develop infant foods (beikosts) with a goal of achieving low amounts of antinutritive substances and high trace element content. The results were subjected to response surface methodology and the authors verified that carrot was the main source of tannins in beikosts and was the key factor in controlling antinutritive substances, whereas rice and potato were key ingredients for controlling phytic acid content in the formula. None of the vegetable ingredients exerted major effects on trace element content in the final product, with the exception of a significant effect of rice on manganese content and pea on copper content. From this study, it is possible to state that the development of foods with special nutritional requirements is feasible by using a statistical approach.

STATISTICAL PACKAGES

In order to design and analyse experimental data, there are some free (the well-known are R and Action for Microsoft Excel) and commercial statistical packages, such as SAS (Statistical Analysis Software), SPSS (Statistical Package for Social Science), Statistica, Statgraphics, Minitab, Design-Expert and Prisma, among others. Among these, Minitab and Statistica are the most used packages for design of experiments. They both have a friendly interface, although Statistica seems more complete and has a magnific graphics output. Action software, developed by Brazilian scientists, is also free to download and presents the DOE features. This software also has suitable graphics output and is the first statistical system that utilizes the R platform together with Microsoft Excel.

FINAL REMARKS AND PERSPECTIVES

The use of DOE and RSM for food development and process optimization in Food Science and Technology has increased in the last 10 years. In this paper, some types of experimental designs and examples of how DOE and RSM may be applied in microbiology, sensory tests, process optimization and nutrition were reported to provide experimental information to future experimenters. Within this context, the authors believe that readers can take into consideration this information in order to build and to analyse experimental designs to help them to obtain the right answers for their problems.

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