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Experimental Design and Optimization of Ultrasound Treatment of Food Products

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What is the Purpose of Experimental Design?

When scientists or manager in big food industries wants to develop or to modify some process in food production, one must go through lot of experimental and laboratory procedure and system scale up to obtain desired product or process. The development of food products or processes is a complex, expensive and risky multistage process, and special requirements should be considered in this process such as consumer demands, technical conditions and legislation background. Also, the quality of the ingredients, quantity, price, properties and the formulation of the final food also play very important role. Special interest is focused on power ultrasound processing. It can be exploited in several food processing like ultrasound assisted extraction of bioactives, oils, and essential oils, inactivation of microorganisms, enzymes, preservation of aromas, color, etc. In order to formulate and test ingredients that will be used, many food processing indutries use statistical approaches such as response surface methodology (RSM) in their research department in order to achieve the best formulation in relation to sensory acceptance, shelf life, nutritional demands, and physicochemical stability of product. RSM has important application in the design, development and formulation of new products, as well as in the improvement of existing product design. Therefore, technological unit operations and system set up can also be optimized, decreasing thus the volume of experiments and ingredients, time, chemicals, total financial input, energy etc.

Therefore, it is very important nowadays, to use experimental design to save chemicals, experimental material, time and energy and to produce environmentally friendly procedure. OMICS group, and its Journal of Food Processing and Technology in periodical issues emphasize and promote green technology and processes to be used in food processing and technology. One of these technologies is power ultrasound.

Experimental design of power ultrasound processing of food products

Mathematical modeling for food product development or unit operations modification to produce a food is increasing and adopting some statistical techniques, such as response surface methodology (RSM), to solve problems where several independent variables (or factors) influence the response variable value. Technical problems, data analysis and modeling, experimental design and independent variables' choice make food scientist and developers harder engagement. Variable selection and optimization of outcome of desired product is challenging task. For this purpose, scientist should aim at determining optimum levels of the main ingredients/compounds in order to obtain suitable responses from desired properties (like aromas, color, physicochemical, rheological and sensory parameters). Therefore, by using statistical techniques, ingredients and their variation range can be tested with a minimum number of experiments while reducing energy, time and cost of testing. This procedure is crucial when laboratory testing of power ultrasound processing of food product should be scaled-up. Scientist that deals with this area of novel non-thermal food processing knows the problem of scaling up, and the procedure is mostly not linear when comparing results from small volumes to large ones. Therefore, experimental planning and design would lead to energy, time and consumables saving, and to be environmentally friendly.

Power Ultrasound

High-intensity ultrasound is used in many food applications, such as emulsifying, sterilizing, extracting, degassing, filtrating, drying, and enhancing oxidation. Ultrasound represents mechanical waves, i.e. a variation of pressure or density with frequencies above the human hearing threshold (ca. 18 kHz). High intensity ultrasound generated by periodic mechanical motions of a probe transfers ultrasonic energy into a fluid medium and triggers extremely high alterations in pressure leading to the formation of small rapidly growing bubbles (cavities), which expand during the negative pressure excursion, and implode violently during the positive excursion generating high temperatures, pressures and shear forces at the probe tip. These resultant micro bubbles collapse violently in the succeeding compression cycles of propagated ultrasonic waves. This results in localized high temperatures up to 5000 K, pressures up to 50,000 kPa, and high shearing effects. Consequently the intense local energy and high pressure bring about a localized pasteurization effect without causing a significant rise in macro-temperature. Also, ultrasound processing enhances the extraction of phenolic and other bioactive compounds from grape must or wine, fruits, essential oils from herbs or oils from oil seeds. Power ultrasound could be used in inactivation of microorganisms and enzymes. The application of ultrasound in the assisted extraction is just one example where experimental design can be used. Factors that can be independent variables could be (amplitude of ultrasound, treatment time, temperature and volume of samples, diameter of probe, composition of extraction fluid (also chemicals can be bypasses) etc. Also, drying processes can be designed and independent factors can be varied (amplitude of ultrasound, thickness of samples, diameter of sample, temperature of process, size and type of sliced samples, speed and type of circulating hot air etc.). Also, ultrasound could be used as pretreatment prior drying of fruits and vegetables. For this process, also factor could be varied (osmotic dehydration, composition of osmotic liquid, temperature and its volume etc.).

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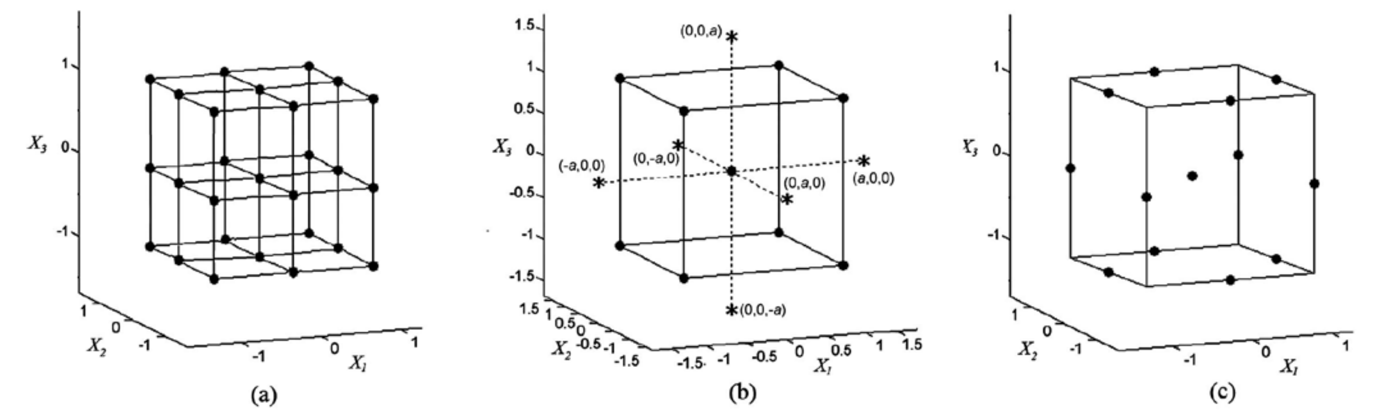

Figure 1: Response surface design for three factors: (a) three-level full factorial design; (b) Box-Wilson central composite design and (c) Box-Behnken design in graphics.

Thus, the results (sensory, rheological, chemical, physicochemical, etc.) may be studied, interpreted and also optimized with these statistical techniques, resulting in a product with enhanced features and, therefore, more likely to be accepted by the target public.

Optimization of experimental design

In order 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 continues until all factors have been analyzed. Besides being laborious, this procedure is erroneous, as it does not take into account the interaction between factors. Ideal level of a factor was determined based on certain levels of other factors. This procedure is often resulting in a different and wrong value for the response variable.

Therefore factorial design (central composite design- CCD) should be used in order to determine the effect of one by one factor and the most important their interactions. Input variable could be amplitude of ultrasound, volume of sample, thickness of samples, diameter of sample, diameter of probe, pulse of ultrasound, temperature of process, size and type of sliced samples, speed and type of circulating hot air, percentage of protein, treatment time, percentage of solvent, power etc. Therefore, all factors must be simultaneously varied, with a minimum number of assays, according to the design methodology. The major disadvantage of this technique is that it does not include interactive effects among the variables and, eventually, it does not depict the complete effects of the parameters on the process. In order to overcome this problem, optimization studies using response surface methodology (RSM) can be performed to obtain optimum conditions. Response variables are our process results (yield of oil, amount of extracted bioactive component, anthocyanins content, essential oils, drying kinetics, aroma compounds, rheological properties, sensory evaluation, density, emulsifying and foaming properties of proteins, color, log CFU/mL, total number of microorganisms, thermal properties, texture profile, solubility of proteins, activity of enzymes etc.). Each and every output results could be optimized, that means that we can determine at each individual factor are we having the highest amount of aroma compounds, best color values, best drying characteristics, best foaming and emulsifying properties, highest extraction yield, highest amount of phenolics, anthocyanins, best rheological properties, shortest drying time, least number of total microorganisms, lowest or highest enzymatic activity (based on the purpose), cheapest solvent that has statistical significant output in extracted compounds, least oxidation etc.

The full factorial design is applied when the purpose is to determine which factors (independent variables) are important in the study, and to determine the range of values (levels) of these factors. Also, in order to visualize the effect of different factor combinations on the selected response variables. When there are many factors to be studied and there is not much time, neither raw material, 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. RSM is useful tool to optimize the ultrasound extraction conditions, drying process, pasteurization conditions, inactivation of enzymes, foaming condition etc., increasing yield, reducing drying time, solvent input and reducing costs.

For two-level factorial designs (2k), the mathematical model used to describe the relationship between factors and the response variable is linear. However, in several studies, there is an interest in determining which factor levels take 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 (process variables) (i.e. amplitude of ultrasound, temperature of sample, treatment time or sample size, hot air velocity, thickness of sample) or with a central composite design - CCD; in both cases, the parabola is a mathematical model that accomplishes this objective. The three-level design (3k) is not the most efficient way to model a quadratic relationship; the response surface design is preferred, and requires lesser assays to achieve a better modeling.

Response surface methodology (RSM) 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 $x_1, x_2, \ldots, x_k$. The first step in using this methodology is to determine a mathematical relationship between the response variable and the independent variables. 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, should be applied

$$y = \beta_0 + \Sigma_{i=1}^{k} \beta_i x_i + \Sigma_{i=1}^{k} \beta_{ii} x_i^2 + \Sigma_{i<j} \Sigma_{j=2} \beta_{ij} x_i x_j + \varepsilon$$

RSM is very useful to determine a statistical significance of all factors whose levels are represented by $x_1, x_2, \ldots, x_k$; (amplitude of ultrasound,

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treatment time, temperature and volume of samples, diameter of probe, composition of extraction fluid, thickness of samples, diameter of sample, temperature of process, size and type of sliced samples, speed and type of circulating hot air, osmotic dehydration time, composition of osmotic liquid, temperature and its volume etc.). Also, to establish a relationship between responses (y) (yield of oil, amount of extracted bioactive component, anthocyanins content, essential oils, drying kinetics, aroma compounds, rheological properties, sensory evaluation, density, emulsifying and foaming properties of proteins, color, log CFU/mL, total number of microorganisms, thermal properties, texture profile, solubility of proteins, activity of enzymes etc.) and process variables $x_1, x_2, \ldots, x_k$ that can be used to predict response values for a given set of control variables; 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.

Conclusion

Experimental design and optimization of ultrasound processing of food product can be very efficient tool in order to conduct process, change existing process, and design a new product or to scale up. It is very efficient tool to determine optimum conditions of treatment, to reach desired minimum or maximum output values, to save chemical, raw material and consumables, lab time, money and to save energy and to protect the environment.