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Black tea cream effect on polyphenols optimization using statistical analysis

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ABSTRACT

Black tea cream formation is an inhibitor for the polyphenols separation since it decreases the amount of available polyphenols. Four factors that are considered to have an impact in the amount of tea cream and polyphenols availability are studied: temperature, amount of solids, pH and amount of EDTA.

By using a design of experiments instead of a one-factor-at-a-time, additional information such as interaction effects can be obtained. The objective is to determine the optimum combination range for the factors that minimize the cream formation, while maximizing the amount of polyphenols in the clear phase.

Statistical analysis is used to determine which factors significantly influence the responses and to generate polynomial models. This is a very effective tool and it indicates that EDTA is the only non-relevant factor. The optimization results in a 37% increase in the yield of theaflavins and a 20% increase in the yield of catechins.

1. Introduction

Black tea polyphenols are formed during the fermentation step (controlled enzymatic reactions) of green tea leaves. While in green tea mostly catechins can be found, black tea is the source of several types of polyphenols formed by enzymatic polymerization of catechins, including theaflavins (Fig. 1), which can only be found in black and oolong teas (Harbowy & Balentine, 1997; Yang, Chung, Yang, Chhabra, & Lee, 2000). Several studies demonstrate that populations with high consumption of plant-based foods, have a lower incidence of cardiovascular diseases and certain types of cancer, which may be related to the fact that plant-based foods containing polyphenols have an antioxidant potential (Shahidi, 2007).

During the tea extraction process part of the compounds that are soluble in hot water, turn out to be insoluble in cold water and form a precipitate, which is referred to as tea cream. This temperature dependent solubility is particularly relevant in the case of black tea, when compared to other types of teas.

Although the tea cream detailed formation mechanism is not known, it is considered to be caused by interpolymer complexation. The cream formation occurs due to the change in molecular weight and solubility of phenolic polymers upon complexation with several other components, e.g. proteins, polysaccharides, lipids and metal cations. The tea cream phase contains mainly polyphenol–protein complexes, while pectin–polyphenol complexes are found in the clear phase (Tolstoguzov, 2002).

A range of variation to avoid the formation of black tea cream was already determined for two factors: amount of solids in the tea solution and the temperature (Penders, Scollard, Needham, Pelan, & Davies, 1998). The authors found that the phase diagrams for black tea have some analogy with the phase behavior of mixtures of simple compounds. These types of mixtures have one phase at high temperatures, but separate into immiscible phases below an upper critical solution temperature.

There are, however, other factors like pH and chelating agents that can influence the extent of cream formation and the amount of polyphenols in each existing phase (Jobstl, Fairclough, Davies, & Williamson, 2005; Tolstoguzov, 2002; Wu & Bird, 2010).

The vast majority of previous studies about the tea cream effect focus on achieving a complete tea cream formation (using low temperatures), to be able to characterize this phenomenon. However, in this work, the stage of full cream formation is never reached, since one of the goals is to minimize the cream formation, process systems engineering (PSE) is an educational and research discipline within the chemical engineering that includes design, modeling, optimization, control and analysis of process systems. In this work

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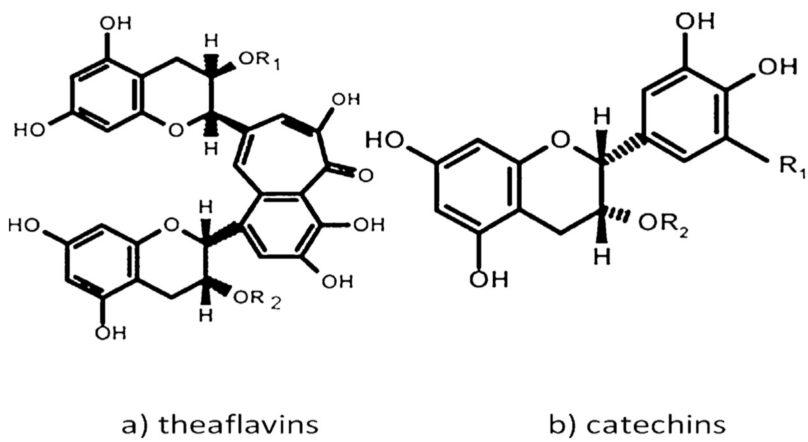

Fig. 1. (a) Theaflavins structure and (b) catechins structure.

we applied as tools a systematic design of experiments (DoE), statistical analysis and optimization, which fit within the PSE activities.

In this work a design of experiments has been setup to fully characterize the selected design space with polynomial models, with the objective of minimizing the cream formation and maximize the amount of polyphenols in the clear phase. The resulting models can be used to optimize the operational conditions (responses) and can also be extrapolated to the outside of the design space.

In addition, although this type of experimental design with statistical analysis, modeling and optimization are widely used within the PSE community, they are not commonly applied within the food industry. The more conventional approach for studying the factors influencing the tea cream formation is to study one-factor-at-a-time (OFAT). However, a DoE allows a much better relative efficiency, i.e. covers a broader design space and reveals interactions between various factors, providing a more detailed level of knowledge about the process.

The variation interval for the four selected influence factors; temperature (*T*), amount of solids, pH and concentration of chelating agent (EDTA) is selected based on literature. The influence of these factors is tested to assess if they have a significant effect on the availability of theaflavins and catechins in the clear phase. As a result of insufficient information available in the literature, screening experiments with varying pH and EDTA concentrations are necessary to determine the proper variation ranges.

In order to evaluate the DoE objectives four responses are taken: cream split factor (C_{sf}), yield of solids (Y_s), yield of theaflavins (Y_{tf}) and yield of catechins (Y_{cat}). As it is not known whether the influence factors have linear or nonlinear behavior, a response surface methodology (RSM) and a Box–Behnken design are proposed for modeling and optimization of the influence of some operating variables. The RSM can capture linear effects as well as nonlinear (quadratic effects). Statistical tests for significance and the development of quadratic relationships that link the influence factors to the responses, can be used to optimize the process, i.e. to find settings that minimize the cream formation and maximize the amount of polyphenols recovered.

2. Theory

The response surface methodology (RSM) includes several mathematical and statistical tools that can model and optimize a response of interest of an arbitrary experiment as a function of a selected set of variables:

$$y = f(d_1, d_2, \ldots, d_i) \qquad (1)$$

where y is the quantity of interest, called the response, d_i are the variables (factors) that affect the outcome of the experiment and f is the mathematical function that describes the influence of each factor on the final response.

The objective of the RSM is to find the best expression for the function f, while minimizing the number of experiments. The function f is a polynomial series that can be represented in the following form (Montgomery, 1997):

$$y = a_0 + \sum a_i d_i + \sum a_{ij} d_i d_j + \sum a_{ii} d_i^2 + \cdots \qquad (2)$$

where d_i, d_j are the independent variables and a_0, a_i, a_{ij} and a_{ii} are constants.

In this work, the response is fitted to a second order model that represents the correlation with the independent variables. The selected RSM design is the Box–Behnken design. This design is constructed by combining two-level factorial designs with incomplete block designs and has a complex confounding of interaction. The experimental points are placed on the edges of a *N*-dimensional hypercube.

The DoE experiments are generated using the Statgraphics software. A total of 27 experiments (4 factors and 3 center-points replication) are collected in Table 1, where each experimental factor (A: amount of solids, B: pH, C: temperature and D: EDTA) is varied in 3 levels (−1, 0, 1).

Center-points are additional experimental runs in a point midway between the low and high level of all the factors. The centerpoints are many times the only replicated experiments in an experimental design, providing information about the reproducibility of the process. The experiments are generated in a randomized way, to reduce the effect of lurking variables.

In the DoE, the analysis of variance (ANOVA) compares the variation within replicated runs with the residual (model error) variation and analyses the significance of the experimental data. This analysis considers that if the factor effect is larger than the experimental error, the changes in the response are indeed effects from the influence factors. The output includes the Fisher's *F*-test (overall model significance) and the Student's *t*-value for the estimated coefficient (Zondervan, Zwijnenburg, & Roffel, 2007).

2.1. Factors

As mentioned before four factors are chosen for the DoE; A: amount of solids, B: pH, C: temperature and D: EDTA. Besides selecting these four influence factors it is also necessary to set the DoE design space, by choosing a variation range for each factor (Table 2). The following ranges are selected: