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Efficacy of response surface methodology in optimization the extraction of annatto seed's colorants

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Introduction

Food consumers tend to use natural products without any synthetic additives. Therefore, many studies have been conducted to investigate the possibility of replacing synthetic additives with natural substances in various food products.

Annatto dye is a natural carotenoid pigment extracted from the pericarp of *Bixaorellana*L. seeds. The major fraction of the annatto extract is 9′-cis-bixin that is soluble in oil and 9′-cis-norbixin is the major dye fraction of the alkaline extract that is soluble in water. Annatto dye creates orange to red color in food and to be used as a natural pigment in a variety of food materials including cheese, butter, margarine, confectionary and bakery products, different kinds of drinks, snacks and jams. In addition, annatto dye has antioxidant and antimicrobial activity.

Nowadays, the extraction of natural dye from plant resources has become a common technology. However, complementary information using new methods and optimization of the extraction conditions seems to be necessary in order to accomplish the highest yield of extraction. Response surface method (RSM) is effective and efficient in optimizing color extraction conditions.

In this study, the different conditions of extraction process were optimized through RSM in order to obtain maximum yield and best quality of annatto dye.

Materials and methods

Materials

Annatto seeds were purchased from Hyderabad, India. All solvents were analytical grade, Merck, Germany.

Extraction of annatto dye

A certain amount of annatto seeds was soaked in n-hexane for 6 hours in order to remove oils. After filtration, the defatted seeds were used for dye extraction. Since chloroform and acetone showed the highest yields of extraction during preliminary experiments, these two solvents and their mixtures were exploited for the final experiments assigning 0 for pure acetone and 100 for pure chloroform. The extracts were filtered through Whatman filter paper NO.1 and then vacuum-dried in the 1410D-2E vacuum oven (Shel Lab, USA) to produce dye powder. Low temperatures (40°C) were applied to prevent thermal dissociation of conjugated double bonds during drying.

Dye measurement

The coloring strength was measured according to Vasu et al. method; model UV-160A spectrophotometer Shimadzu, Japan, at 502 nm in which bixin has the maximum absorbance value when it is dissolved in chloroform.

Determination of extraction efficiency

The obtained powder was weighed and the mass ratio of the powder to the weight of the seeds was taken into account as the extraction yield.

Experimental design

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In this study, Minitab® software version 16.1.1 (Minitab Inc. USA. 2010), was used and a five level four factor central composition design was created to investigate the effect of the independent variables such as temperature, extraction time, seed to solvent ratio and chloroform concentration on the dependent variables namely the extraction efficiency and absorbance values.

Results and Discussion

The values of R^2, R^2-adj and R^2-pred revealed that the full quadratic models were the most adequate for the extraction efficiency and absorbance values.

The all of the linear terms show a significant effect except the extraction time ($P< 0.05$). The quadric term of extraction time and the seed to solvent ratio also had a significant effect ($P< 0.05$) on the extraction efficiency, however, the effect of other two quadric terms was insignificant ($P> 0.05$). The interactive terms of extraction temperature* seed to solvent ratio(X_1X_3) and the seed to solvent ratio*Chloroform concentration (X_3X_4) had a significant effect on the extraction efficiency ($P< 0.05$); however, the other two interactive terms was insignificant ($P> 0.05$).For the absorbance values, the all of the linear terms show a significant effect ($P < 0.05$); the quadric term of extraction temperature (X_1^2) and the seed to solvent ratio (X_3^2) also had a significant effect ($P< 0.05$) on absorbance values, but, the effect of other two quadric terms (X_2^2 and X_4^2) did not show a significant effect ($P> 0.05$). The all of interactive terms was insignificant ($P> 0.05$).

An increase in the extraction efficiency was observed with the increasing temperature. Banik and Pandey while extracting oleanolic acid from *Lantana camara*roots demonstrated that as temperature increases extraction efficiency improves too. However, at temperatures higher than 70 °C, the annatto seed pigments were degraded and the response was reduced so the quadratic effect of temperature was negative.

The absorbance value was increased by increasing the temperature; however, the absorbance value decreased at higher temperature by thermal decomposition and damage of the conjugate double bond.The absorbance value increased by increasing the chloroform concentration and seed to solvent ratio initially, however, subsequently decreased due to the damage of the conjugate double bond in higher chloroform concentration and saturation of solvent in higher seed to solvent ratio.

Temperature of 48.33 ˚C, extraction time of 2 hr, the ratio of seed to the solvent of 12.88 and chloroform concentration of 100% were found to be as the optimum conditions of the process. The extraction efficiency of 3.95 percent of annatto seed and absorbance value of 0.597 were acquired as the predicted results.

Keywords: Extraction, Annatto seed, Optimization, Colorant, Response surface methodology