Industrial Crops and Products 41 (2013) 165–171

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Modeling of microwave-assisted extraction of natural dye from seeds of *Bixa orellana* (Annatto) using response surface methodology (RSM) and artificial neural network (ANN)

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ARTICLE INFO

Article history:
Received 29 January 2012
Received in revised form 1 April 2012
Accepted 5 April 2012

Keywords:
Natural dye
Bixa orellana seeds
Microwave-assisted extraction
Response surface methodology
Central composite design
Artificial neural network

ABSTRACT

With ever increasing demand for eco-friendly, non-toxic colorants, dyes derived from natural sources have emerged as a potential alternative to relatively toxic synthetic dyes. In the present work, microwave-assisted extraction of yellow-red natural dye from seeds of *Bixa orellana* (Annatto) was studied. Response surface methodology (RSM) and artificial neural network (ANN) were used to develop predictive models for simulation and optimization of the dye extraction process. The influence of process parameters (such as pH, extraction time and amount of Annatto seeds used in extraction) on the extraction efficiency were investigated through a two level three factor (2^3) full factorial central composite design (CCD) with the help of Design Expert Version 7.1.6 (Stat Ease, USA). The same design was also used to obtain a training set for ANN. Finally, both the modeling methodologies (RSM and ANN) were statistically compared by the coefficient of determination (R^2), root mean square error (RMSE) and absolute average deviation (AAD) based on the validation data set. Results suggest that ANN has better prediction performance as compared to RSM.

1. Introduction

Since time immemorial, man has always been fascinated by the beautiful and awesome spectacle of the colors of nature. In primitive times, humans exploited natural resources, particularly flora and fauna, for the extraction of dyes and colorants: mainly for coloration of textile fibers and decoration of their caves and dwellings, thereby marking the beginning of a colorful life style (Singh and Arora, 2011). Natural dyes were extensively used by the civilizations of Ancient China, Egypt and the Middle East (Saha, 2010; Gupta and Suhas, 2009). Natural dyes have also been a part of India's ancient heritage. Natural colors were used to dye the exotic fabrics from ancient India and these commanded a premium in the markets of the world. Colors extracted from plants and fruits were used in the exotic cave paintings and frescoes of Ajanta and Ellora (Aurangabad, Maharashtra, India). Till the late nineteenth century, almost all commercial dyes were extracted from plants, insects and mollusks (Gupta and Suhas, 2009).

In 1856, the accidental discovery of synthetic dye (Mauveine) by English chemist Sir William Henry Perkin triggered a marked decrease in the wide and exclusive use of natural dyes for coloration of materials (Leitner et al., 2012; Singh and Arora, 2011). Synthetic dyes were much easier to manipulate and were judged "better" in any aspect of use, such as brilliance and range of color, durability and costs (Leitner et al., 2012; Zarkogianni et al., 2010). Natural dyes still lasted longer on the cloth when exposed to water and light, but convenience won out, and by the early 1900s the traditional natural dyes had almost entirely fallen by the wayside.

At present, more than 100,000 dyes are available commercially. Worldwide, nearly 1 million tonnes of synthetic dyes are produced annually, of which India's contribution alone is 60,000 metric tonnes (Singh and Arora, 2011). Synthetic dyes are widely used in a number of industries such as textile, leather, paper, printing, food, cosmetics, paint, pigment, petroleum, solvent, rubber, plastic, pesticide and wood preserving chemicals (Chowdhury et al., 2011). During textile/leather processing, inefficiencies in dyeing result in a large amount of dyestuff being directly lost in the wastewater, which ultimately finds way into the environment. It is estimated that 10–35% of the dye is lost in the effluent during the dyeing process (Sivakumar et al., 2009a). The release of colored effluent wastewaters into the aquatic ecosystem represents both environmental and public health risks because of their negative ecotoxicological effects and bioaccumulation in wildlife (Chowdhury and Saha, 2010; Saha et al., 2010). Such wastewater streams can be treated using suitable adsorbents, but creates potential disposal problems (Sivakumar et al., 2011). Currently, there is

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http://dx.doi.org/10.1016/j.indcrop.2012.04.004

no effective technology available for removal of these toxic dyes from effluents (Sivakumar et al., 2011). With ever increasing environmental concern on the use of synthetic dyes, natural dyes appear to be the most appropriate substitute to the relatively toxic synthetic dyes. Natural dyes are not an innovation but a revival of a rich and prudent tradition.

Natural dyes derived from flora and fauna are considered to be safe because of their non-toxic, non-allergic, non-carcinogenic and biodegradable nature (Ali et al., 2009; Sinha et al., 2012a). They do not create any environmental problems at the stage of production or use and maintains ecological balance (Sinha et al., 2012b; Sivakumar et al., 2011; Ali et al., 2009). Today, the global demand for natural dyes in nearly 10,000 tonnes which is approximately 1% of the synthetic dyes consumed worldwide (Sivakumar et al., 2011). The demand is expected to grow rapidly in the near future.

Most natural dyes can be obtained from different plant parts such as roots, bark, leaves, flowers, fruits and seeds. However, plants generally give less amount of coloring matter on extraction with water. Since the coloring component is tightly bound to the cell wall, there is a need for novel techniques to improve the major mechanism of natural dye extraction such as rupture of cell wall, release of natural dye and transport of dye into the external medium (Sivakumar et al., 2011). In recent years, researchers worldwide have investigated the efficacy of different techniques for extraction of natural dyes from different plant parts. Ali et al. (2009) studied the alkaline extraction of natural dye from Henna leaves. Sivakumar et al. (2009b) used ultrasound in the extraction of vegetable tannins. They also used ultrasound in the extraction of natural dye from beetroot, pomegranate rind, bark of *Acacia decurrens* and flowers of *Tagetes erecta*, *Mirabilis jalapa* and *Celosia cristata* (Sivakumar et al., 2009a, 2011). Leitner et al. (2012) formulated a solid dyestuff product from aqueous extracts of dried Canadian Goldenrod plant material. Our recent studies show significant improvements in extraction of natural dyes from pomegranate rind and *Butea monosperma* flowers due to the use of microwave (Sinha et al., 2012a,b). Microwave enhances the mass transfer of coloring matter from natural plant material and transport to the solvent medium. In this regard, the present paper aims at microwave-assisted extraction of natural dye from seeds of *Bixa orellana*.*B. orellana* (Annatto) is a perennial tree, 3–9 m high, native to tropical America, but widely cultivated in Africa and other tropical countries: Bolivia, Brazil, Dominican Republic, Ecuador, Guyana, India, Jamaica, Mexico, Peru, Sri Lanka and Suriname (Rivera-Madrid et al., 2006; Germano et al., 1997; Preston and Rickard, 1980). The plant is commercially important for its seeds, which contains large amounts of a yellow-red pigment – (a mixture of carotenoids such as bixin, norbixin, β-carotene, cryptoxanthin, lutein, zeaxanthin and methylbixin) (Alves de Lima et al., 2003). The pigment is primarily used for coloring cheese and other dairy products (butter, ice-cream, margarine, snacks, etc.), and body care products (Guiliano et al., 2003; Jondiko and Pattenden, 1989). At present, the average annual worldwide production of Annatto seeds is 10,000 tonnes. Latin America produces 60% of the world's Annatto, followed by Africa (27%) and Asia (12%) (Guiliano et al., 2003). The large Annatto cultivation guarantees its stable supply, thereby suggesting extracts from Annatto seeds to be an attractive and convenient source of natural dye. The present work was, therefore, undertaken to extract coloring component from seeds of Annatto. Recently response surface methodology (RSM) and artificial neural network (ANN) methods have been used jointly for both modeling and optimization of a microwave-assisted extraction method (Moghaddam and Khajeh, 2011). Therefore, in the present study, a two level three factor (2^3) full factorial central composite design (CCD) in RSM and an ANN based models were developed to predict the relationship between the experimental variables (pH, extraction time and amount of Annatto seeds used in extraction) on the total amount of dye extracted (response variable). Finally, the optimal solutions offered by RSM and ANN were statistically compared by the coefficient of determination (R^2), root mean square error (RMSE) and absolute average deviation (AAD) based on the validation data set. To the best of our knowledge, this is the first report comparing RSM and ANN in natural dye extraction technology.

2. Materials and methods

2.1. Annatto seeds

Annatto seeds, used in this study, were collected fresh from our Institute garden. The seeds were washed with double distilled water to remove any adhering soil and dust, dried at room temperature and stored in dark.

2.2. Experimental procedure

Microwave-assisted extraction was performed in an experimental microwave oven (Samsung, Model PG-3200, Korea) with the power of 900 W and the frequency set on 2450 MHz. Typically a weighted amount of dry seeds was subjected to extraction by adding 50 mL double distilled water in a 250 mL glass beaker. The pH was adjusted to the desired value with 0.1 M HCl and 0.1 M NaOH solutions using a digital pH meter (LI 127, ELICO, India) calibrated with standard buffer solutions. The glass beaker was placed in the center of the microwave oven, containing a circular, 360° rotating carousel with different durations of exposure. The beaker was covered with a watch glass to prevent loss of water by evaporation. After microwave heating, the mixture in the glass beaker was allowed to cool down to room temperature and filtered using filter paper (Whatman, 595-1/2 folded, 110 mm diameter). The filtered extract was then dried using a desiccator. Finally, the extract was analyzed by measuring the optical density at maximum wavelength (λ_{max}) using a UV/VIS spectrophotometer (U-2800, Hitachi, Japan).

2.3. Experimental design

A number of factors such as solvent pH, extraction time and the amount of Annatto seeds subjected to extraction can significantly affect the dye extraction efficiency. Therefore, a standard RSM design (CCD) was used to identify the relationship between the response function (total amount of dye extracted) and the process variables (solvent pH, extraction time and amount of Annatto seeds used in extraction). The experimental range of the selected process variables with their units and notation is given in Table 1. The response variable, T_{dye} (total amount of dye extracted, mg L^{-1}) can be expressed as a function of the independent process variables according to the following response surface quadratic model:

$$T_{dye}\ \ (\text{mg L}^{-1}) = \beta_0 + \sum_{i=1}^{k}\beta_i x_i + \sum_{i=1}^{k}\beta_{ii}x_i^2 + \sum_{i=1}\sum_{j=1+1}\beta_{ij}x_i x_j + \varepsilon \quad (1)$$

where, β_0 is the constant coefficient, β_i, β_{ii}, β_{ij} are the coefficients for the linear, quadratic and interaction effect, x_i and x_j are the independent variables and ε is the error.

A total of 20 experiments were performed in duplicate according to the CCD matrix in Table 2 and the average values were used in data analysis. The experimental data were analyzed by the software, Design Expert Version 7.1.6 (Stat-Ease, USA). The adequacy of the developed model and statistical significance of the regression coefficients were tested using the analysis of variance (ANOVA). The interaction among the different independent variables and their