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Extraction of lutein from Marigold flower petals – Experimental kinetics and modelling

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Abstract

The extraction kinetics behaviour of lutein from Marigold flower petals and simultaneous alkali hydrolysis has been studied. Extraction was carried out by varying following operating conditions: type of organic solvent, temperature, ratio liquid: material, concentration of alkali solution, and particle size of plant material. Experimental extraction curves were analysed with a mathematical model derived from Fick's second law. The extraction of lutein appeared to be governed by slow and fast diffusion processes. Results showed that the intra-particle diffusion was the rate-governing step of the extraction process, and that the chosen model gives very good approximation of experimental data.

Keywords: Lutein; Marigold; Conventional extraction; Diffusivity; Mass transfer

1. Introduction

The principal natural colorants used in modern manufacture are anthocyanins, betalains, carotenoids, chlorophylls, riboflavin and caramel. Carotenoids are responsible for the yellow, orange and red pigments in a large variety of plants and animal kingdoms, including carrots, red tomatoes, paprika, annatto, saffron, palm oil, corn kernels, Marigold petals and red salmon, trout, shrimps, crabs, lobsters (González de Mejía, Loarca-Pina, & Ramos-Gomez, 1997; Hendry, 1996). Marigold is a stout branching annual with large yellow to orange flower heads which prefers a warm, low humidity climate and is as easily cultivated ornamental and medical plant, originating from Latin America, widespread all over the world with numerous species (Piccagliy, Marotti, & Grandi, 1998; Zorn, Breithaupt, Takenberg, Schwack, & Berger, 2003). Its flower petals are an excellent and most important source of carotenoids, the yellow carotenoids such as carotenes (α- and β-carotene) and xanthophylls (lutein, zeaxanthin) as well as red carotenoids such as capsanthin, canthaxanthin and astaxanthin (Handelman, 2001).

Lutein is one of the major constituent of green vegetable, orange fruits and egg yolk, where it exists in its free – nonesterified form. The most important source (more than 20-times higher amount) are flower petals of Marigold, where lutein is chemically bound to various types of fatty acids such as lauric, mystric and palmitic acids. Upon saponification of the Marigold extract, the lutein fatty acid esters are converted to free lutein (Khachik, 1995). Commercially, lutein isolated from Marigold flowers (*Tagetes erecta*) was first used in chicken feed to provide a yellow colour to the skin of broilers and yolks of layers. Its field of application has been as an excellent antioxidant enlarged on nutritional, cosmetic and pharmaceutical industry. It is reported in the literature that, the risk of chronic disease, such as heart disease, cancer and age-related eye diseases might be significantly reduced by diets rich in lutein (Khachik, 1995; Madhavi & Kagan, 2002; Rodriguez, Torres-Cardona, & Diaz, 2001).

Although chemical processes for the synthesis of xanthophylls from commercially available starting materials are known, such processes are extremely time consuming. They involve multiple steps, and have not provided an economical process for the production of xanthophylls (Khachik, 1995). A more economical route for the large-scale production of substantially pure xanthophylls is the traditional commercial

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process that extracts the oleoresin from Marigold flowers, converts the xanthophylls from the acylated form to the free form by saponification (alkali hydrolysis) in two separated steps, and at last purifies the impure lutein as it is contaminated with numerous chemical impurities in multi-step procedure of purification and sometimes additional recrystallization (Ausich & Sanders, 1997; Khachik, 1995; Madhavi & Kagan, 2002; Rodriguez et al., 2001).

The dynamic behaviour of Marigold oleoresin extraction was so far observed only for high-pressure extraction technique using liquid and supercritical carbon dioxide in order to analyse the influence of pressure and temperature on extraction yield of xanthophylls esters (Ambrogi & Egger, 1997; Baumann et al., 2004; Campos, Michielin, Danielski, & Ferreira, 2005). Since the intention of present kinetics study of Marigold extraction was to examine the isolation of free – nonesterified lutein, applicability of one step procedure comprising conventional extraction and simultaneous saponification (alkali hydrolysis) was examined. The only method for this kind of simplified isolation of free lutein has been investigated by Khachik (2001).

The kinetics of solute extraction from natural sources such as plants, seeds, flowers, roots, leaves and other materials consist of releasing solutes from porous or cellular matrices into a solvent phase via mass transfer mechanism (Campos et al., 2005). Most of the mass transfer analyses are based on the assumption that the diffusion is the rate-limiting step and its rate can be approximately predicted by appropriate mathematical solutions of the simplified unsteady state second-order Fickian equation (Shi & Maguer, 2003).

Understanding of mass transfer phenomenon at the solid-liquid interface from an engineering point of view is desired in scaling-up processes from analytical to pilot scale and consequently in the development of industrial applications. The aim of this research work was to examine the interactions of different operating parameters on the extraction of lutein from Marigold flower petals (*T. erecta*) in analytical scale. Therefore, the extraction kinetics of lutein from Marigold under different operating parameters (solvent, temperature, ratios solvent/material and alkali solution/material, concentration of alkali solution and particle size) was studied, which provides a very good basis for further optimisation of the presented process. Furthermore, experimental extraction curves were analysed with mathematical model derived from Fick's second law, and diffusion coefficients of lutein under different operating conditions were determined.

2. Materials and methods

2.1. Materials

Dried Marigold flower petals (*T. erecta*) were donated from Slovenian producers. All solvents used for experimental as well as analytical purposes and chemicals were purchased from Merck (Germany) and Kemika (Croatia), respectively. Authentic standard of xanthophyll-lutein from alfalfa was obtained from Sigma-Aldrich and was 91% pure (Prod. No. X6250).

2.2. Methods

Marigold flower petals were ground and sieve analysis of the ground material was made to determine the particle size distribution: average and median particle size. Grinding was performed on a laboratory scale, in small quantities, so the heating of the raw material was minimal. Moisture content of plant material was determined using Karl Fisher Titrator (Mettler Toledo DL31).

2.2.1. Experimental procedure

The experiments of conventional extraction of lutein esters from Marigold flower petals and simultaneous hydrolysis were performed in a batch extractor composed of a 500 ml round-bottom flask with a three-necked top connected to a condenser, a magnetic stirrer and a boiler. The batch extractor was filled with plant material, extraction solvent and alkali ethanol solution and the content was heated, during constant mixing, to the desired temperature. Extraction solvents used for the isolation of lutein esters were hexane (Hex), petrolether (PE), tetrahydrofurane (THF), acetone (Ac), ethanol (EtOH) and acetonitrile (AcN). Volume of the extraction solvent used per kg of raw material (R_s) was equal to 10 L/kg and in case of hexane R_s of 15, 5 and 2.5 L/kg were additionally examined. Due to addition of alkali solution (potassium hydroxide in ethanol) to solvent for extraction, extraction and hydrolysis took place simultaneously and as a product extract solution containing free lutein was obtained. Three concentrations (C_a) of potassium hydroxide in ethanol were examined: 5, 10 and 15% (w/v). The volume of alkali solution added per kg of raw material (R_a) was equal to 7.5 L/kg. In the case of 10% alkali solution R_a of 11.25, 3.75 and 2 L/kg were additionally examined.

Samples of solution were taken from the extractor at specific time intervals, filtered and analysed spectrophotometricaly on the concentration of lutein in the solution by UV/VIS spectrophotometer. Extractions were stopped when saturated solutions were obtained, noticed from the plots of concentration of lutein in solution vs. time of extraction.

The extraction efficiency of lutein expressed in g of lutein extracted per 100 g of raw material was calculated:

$$w_{\mathrm{Lut}}(\%) = c_{\mathrm{lut}} V_{\mathrm{extr.sol.}} \quad (1)$$

where c_{lut} is the concentration of lutein in g lutein per L of extract solution (g lut./L extr. sol.) and $V_{\mathrm{extr.sol.}}$ is volume of the extract solution (solvent plus alkali solution) per 100 g of raw material (L extr. sol./100 g mat.).

2.2.2. UV/VIS analysis of Marigold extract solutions

Analyses of extract solutions were performed using UV/VIS spectrophotometer. This method was developed based on the results obtained by an HPLC method, which was first applied for the quantification of lutein in Marigold extract samples. The HPLC analyses of several Marigold extracts, prepared with the procedure described above, were performed by Vitiva Company (Hadolin, 2004) at 446 nm and from the