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Extraction kinetic modelling of total polyphenols and total anthocyanins from saffron floral bio-residues: Comparison of extraction methods

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ABSTRACT

Analysis of the extraction kinetic modelling for natural compounds is essential for industrial application. The second order rate model was applied to estimate the extraction kinetics of conventional solid-liquid extraction (CSLE), ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) of total polyphenols (TPC) from saffron floral bio-residues at different solid-to-liquid ratios ($R_{S/L}$)(1:10, 1:20, 1:30, 1:50 g ml^{-1}), ethanol 59% as solvent and 66 °C temperature. The optimum solid-to-liquid ratios for TPC kinetics were 1:20 for CLSE, 1:30 for UAE and 1:50 for MAE. The kinetics of total anthocyanins (TA) and antioxidant activity (AA) were investigated for the optimum $R_{S/L}$ for each method. The results showed a good prediction of the model for extraction kinetics in all experiments ($R^2 > 0.99$; NRMS 0.65–3.35%). The kinetic parameters were calculated and discussed. UAE, compared with the other methods, had the greater efficiency for TPC, TA and AA.

1. Introduction

The dried stigmas of the *Crocus sativus* flower, known as saffron, are used as spice and dye for food preparations. 92.6 g of floral bio-residues are generated per 100 g of flowers as waste material since the stigma represents only 7.4% (w/w) of total weight of the flower of saffron (Serrano-Dìaz et al., 2012). Saffron floral bio-residues composed of sepals, stamens and styles are rich in bioactive compounds (Serrano-Dìaz et al., 2012; Righi, Parenti, Tugnoli, Schenetti & Mucci, 2015; Zeka, Ruparelia, Continenza, Stagos, Vegliò & Arroo, 2015) and recent reports have identified different pharmacological proprieties in their extracts such as antioxidant, antityrosinase, antidepressant, anti-nociceptive, anti-inflammatory and antifungal activity, as well as cytotoxicity against tumour cell lines and arterial pressure reduction (Montoro, Tuberoso, Maldini, Cabras & Pizza, 2008; Serrano-Díaz, Sánchez, Alvarruiz & Alonso, 2013; Serrano-Díaz et al., 2014) . It is well known, that the quality and quantity of bioactive compounds present in a plant depend on environmental conditions, plant growth, harvesting time and various other processing factors such as drying, extraction, separation and purification. Among these, extraction is a basic step to recover the optimum amount of bioactive compounds. In this regard a wide variety of extraction methods for *C. sativus* petals is suggested (Hosseinzadeh & Younesi, 2002; Fatehi, Rashidabady & Fatehi-Hassanabad, 2003; Hosseinzadeh & Ghenaati, 2006; Babaei, Arshami, Haghparast & Danesh Mesgaran, 2014; Khazdair, Boskabady, Hosseini, Rezaee, & Tsatsakis, 2015; Hosseinzadeh & Younesi, 2002; Fatehi et al., 2003; Ahmadian-Kouchaksaraie, Niazmand, & Maskooki, 2016; Ahmadian-Kouchaksaraie, Niazmand, & Najafi, 2016; Ahmadian-Kouchaksaraie & Niazmand, 2017). However, to the best of our knowledge, there are no published data on the application of kinetic modelling to estimate and compare the kinetics of conventional solid–liquid extraction (CSLE), ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) of total polyphenols, total anthocyanins and antioxidant activity from saffron floral bio-residues extracts.

Ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) are advanced extraction techniques. UAE offers several advantages in contrast to conventional extraction, such as high reproducibility in shorter times, simplified manipulation, reduced solvent consumption and lower energy input (Chemat et al., 2017; Ameer, Shahbaz & Kwon, 2017). The mechanism of UAE is ascribed to the phenomenon of acoustic cavitation that causes the swelling of cells or the breakdown of cell walls. This facilitates the diffusion of the solvent into the cellular material, and consequently enhances the extraction efficiency (Chemat et al., 2017; Pingret, Fabiano-Tixier & Chemat, 2013; Toma, Vinatoru, Paniwnyk, & Mason, 2001). Another green extraction method is MAE. This method is based on the use of microwave energy causing molecular motion by ionic conduction and dipole rotation (Mandal, Mohan, & Hemalatha, 2007). The process induces an increase in temperature and pressure that causes changes in the cell structure, improving the penetration of solvent across the sample matrix (Angiolillo, Del Nobile & Conte, 2015). MAE, in comparison to

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conventional extraction, is a cheap and rapid technique, and the extraction time and solvent consumption are reduced (Kaufmann & Christen, 2002).

Ahmadian-Kouchaksaraie, et al. (2016) reported that maximum yield of antioxidant compounds from saffron petals using conventional solid–liquid extraction was achieved with extraction time of 104 min, extraction temperature of 66 °C and ethanol percentage of 59%. However, the effect of solid–liquid ratio was not studied, despite the fact that a well-defined solid-to-liquid ratio ($R_{S/L}$) is crucial in order to permit a good mixing, and thus high diffusion rate of the solute during the extraction process leading to the maximum extraction yield. Furthermore, the use of large volumes of solvent would affect cost-efficiency of the operation.

There is a need for more efficient processes that can increase yields and the overall quality of natural products at a feasible cost. Furthermore, most industrial processes are basically scaled-up from the processes developed at laboratory scale. Analysis of the extraction kinetic modelling of these compounds is essential for industrial application. For these reasons, the aim of the present study is to propose the second-order rate model to describe extraction kinetics of total polyphenols, total anthocyanins and antioxidant activity of the extracts from saffron floral bio-residues. Extraction methods used are conventional solid–liquid extraction, ultrasound-assisted extraction and microwave-assisted extraction at different solid-to-liquid ratios. The comparison of these extraction methods and their corresponding kinetic modelling are first time attempted for saffron floral bio-residues.

2. Materials and methods

2.1. Plant material

Saffron floral bio-residues were collected from the region Friuli-Venezia Giulia, Italy in November 2016, air dried at room temperature and milled by a domestic grinder. The powder obtained was stored at 4 °C until use. The moisture content of dried powder was 5.85 ± 0.12% (w/w).

2.2. Chemicals

Folin–Ciocalteau reagent was supplied by Carlo Erba (Milan, Italy). Methanol, ethanol, 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$), gallic acid, quercetin, cyaniding-3-glucoside, (+)-α-tocopherol, potassium chloride and sodium acetate were purchased from Sigma-Aldrich (Milan, Italy).

2.3. Extraction methods

Temperature (66 °C) and ethanol percentage (59% v/v) suggested as optimal for conventional solid-liquid extraction by Ahmadian-Kouchaksaraie, et al. (2016) to obtain the maximum yield of antioxidant compounds from saffron petals were used for each extraction method tested.

2.3.1. Conventional solid-liquid extraction (CSLE)

Aliquots of 2 g of saffron floral bio-residues powder were extracted with ethanol/water mixture (59: 41 v/v) with different solid to solvent ratio (1:10, 1:20, 1:30, 1:50 g ml^{-1}). The extractions were carried out at 66 °C for 1, 3, 5, 15 and 30 min. Subsequently, extracts were filtered through acetate cellulose membrane (0.65 µm), using Büchner funnel and stored at 4 °C. Extractions were performed in triplicate.

2.3.2. Ultrasound-assisted extraction (UAE)

For the ultrasound-assisted extraction (UAE) experiments an ultrasonic sonifier (Ultrasonic processors UP200St, Hielscher Ultrasonics Gmbh, Teltow, Germany) equipped with a titanium alloy flat tip probe (13 mm diameter) (Sonotrode S26d14, Hielscher Ultrasonics Gmbh, Teltow, Germany) was used. Aliquots of 5 g of saffron floral bio-residues were mixed with ethanol/water mixture (59: 41 v/v) at solid to solvent ratio 1:10, 1:20, 1:30 and 1:50 g ml^{-1} at 66° C in a 500 ml beaker. The beaker and its contents were immersed into a water bath coupled to a temperature controller (Frigiterm, J.P. Selecta, Barcelona, Spain). The probe, submerged about 4 cm under the surface of the mixture, worked at 26 kHz frequency and 200 W (set and displayed in % on the scale of 10–100) for 0.5, 1, 2, 3, 4 and 5 min. Then the extracts were filtered through acetate cellulose membrane (0.65 µm), using Büchner funnel and stored at 4 °C. Each extraction was performed in triplicate.

2.3.3. Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) experiments were performed with a microwave accelerated extraction device (MARS 5, CEM, Mathews, NC, USA) equipped with a digital timer, temperature and power control. Aliquots of 1 g of saffron floral bio-residues powder were mixed with ethanol/water mixture (59:41 v/v) at different solid to liquid ratio (1:10, 1:20, 1:30 and 1:50 g ml^{-1}). The extractions were carried out at 66 °C and 800 W for 0.5, 1, 2, 3, 4 and 5 min. Then, the extracts were filtered through acetate cellulose membrane (0.65 µm), using Büchner funnel and stored at 4 °C. Each extraction was performed in triplicate.

2.4. Analytical methods

2.4.1. Total polyphenols

The content of total polyphenols (TPC) in the extracts was determined using Folin-Ciocalteau reagent. Briefly, the reaction mixture contained 100 µl of extract or solvent, 500 µl of the Folin-Ciocalteau reagent, 4 ml of water and 2 ml of a sodium carbonate-water solution (15% w/v). After 2 h of reaction at room temperature, absorbance was read at 750 nm using US-Vis spectrophotometer (Shimadzu UV-1650, Italy) to calculate TPC. Gallic acid was employed as the standard. A calibration curve was made with standard solutions of gallic acid in the range 50–500 mg ml^{-1} ($R^2 = 0.99$). All analyses were performed in triplicate. The results were expressed as mg of gallic acid equivalent (GAE) per 100 g of dry matter (mg GAE 100 g DM^{-1}).

2.4.2. Total anthocyanins

The total anthocyanins content (y) was determined using the spectrophotometric pH differential method (Rodriguez-Saona & Wrolstad, 2001). This method is based on the anthocyanin structural transformation that occurs with a change in pH (coloured at pH 1.0 and colourless at pH 4.5). Two dilutions of the same sample were prepared using potassium chloride solution (0.025 M) and sodium acetate solution (0.4 M) adjusted to pH 1.0 and 4.5 with HCl, respectively. The absorbance (A) of each solution was measured at 516 (maximum absorption wavelength) and 700 nm using an UV–visible spectrometer (Eq. (1)). Results were calculated using Eqs. (2) and (3), and were reported based on mg of cyanidin 3-glucoside per 100 g of dry matter (mg cyd-glu 100 g DM^{-1}).

$$A = (A_{\lambda 516} - A_{\lambda 700})_{pH1.0} - (A_{\lambda 516} - A_{\lambda 700})_{pH4.5} \quad (1)$$

$$Anthocyanin\ content\ \left(\frac{mg}{L}\right) = \frac{A \cdot MW \cdot DF \cdot 1000}{\varepsilon \cdot L} \quad (2)$$

$$y\left(\frac{mg_{\ cyd-glu}}{100g_{\ DM}}\right) = \left(\frac{V \cdot Anthocyanin\ content}{m}\right) \cdot 100 \quad (3)$$

2.4.3. Antioxidant activity

The antioxidant activity of extracts was evaluated by the total free radical scavenger capacity (RSC) following the methodology described by Espìn, Soler-Rivas & Wichers (2000) with slight modification. Briefly, 10 µl of extract, was added with 1990 µl of fresh methanol