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Extraction of curcuminoids by using ethyl lactate and its optimisation by response surface methodology

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ABSTRACT

Response surface methodology (RSM) was applied to optimise the extraction of curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) from turmeric using ethyl lactate (EL), ethanol and water under mild conditions (magnetic stirring at room temperature). An augmented simplex-centroid mixture design was used to monitor the dependence of the extraction efficiency from the proportions of the three solvents in the extraction medium. HPLC was used to establish the content of curcuminoids in turmeric and in the extracts. Surface plots for the extracted amount of each curcuminoid covering the whole composition domain were generated by interpolation of the experimental data with quadratic canonical polynomial models. The response surfaces of the three curcuminoids are qualitatively similar and the maximum extraction efficiency was obtained with water-EL 30:70 v/v that ensured the almost quantitative recovery of the three compounds from turmeric. While degradation of the three curcuminoids in water at moderate alkaline pH is relatively fast (half-times are between 0.23 and 8.5 h at pH = 8.6), their stability is noticeably greater in EL (half-times are within 21–69 days). Addition of EL to water is also able to inhibit the alkaline hydrolysis of curcumin and its derivatives, their half-times in the water-EL 30:70 v/v, being within 40–70 h at pH = 8.6. The above evidences suggest that EL is a promising solvent for the extraction of curcuminods from turmeric and a suitable medium for vehiculation of these compounds into drugs or foods.

1. Introduction

Turmeric (the dried ground rhizome of *Curcuma longa* L.) has been used since ancient times as a spice, a colouring agent of foods and textiles and, in traditional Indian and Chinese medicine, as a natural remedy for various diseases [1,2]. The yellow-orange polyphenols curcumin (CUR), demethoxycurcumin (DEM) and bisdemethoxycurcumin (BIS) (Fig. 1), known as curcuminoids, are the major bioactive constituents of turmeric. CUR and its derivatives have been the objects of extensive investigation in the field of biology, medicine and pharmacology over the last decades, which have demonstrated their potential therapeutic properties, including anti-oxidant, anti-inflammatory, anti-microbic and anti-cancer [3–6]. Despite of health beneficial effects, low solubility and poor stability of curcuminoids in aqueous solutions represent severe limitations to their application as drugs or functional food additives.

Chemical properties and bioactivity of curcuminoids are strongly influenced by the keto-enol tautomerization equilibria of the central β-diketone moiety [3,5–7]. Several experimental techniques and computational methods suggest that CUR exists mainly in the keto-enol form (Fig. 1) in aqueous media and in organic protic and aprotic polar solvents, while the equilibrium shifts toward the bis-cheto form in non-polar environments, although the enolate is anyway the predominant tautomer [5,6]. The bis-keto tautomer is stable in water only in acidic and neutral aqueous solutions, but is scarcely soluble in this condition. The keto-enol form predominates in alkaline aqueous solutions where solubility slightly increases but fast hydrolysis occurs [8–10]. The noticeable increase of the degradation rate in alkaline aqueous media and the observed complex pH-dependence of stability has been attributed to the progressive dissociation of the enol hydrogen and of the phenolic groups which results in a gradual disruption of the intra-molecular hydrogen bonding responsible for the stabilisation of the keto-enol structure [5,6].

The curcuminoids can be isolated from turmeric by application of various technologies including conventional solvent extraction [1,11], microwave- or ultrasound-assisted extraction [12–15], pres-

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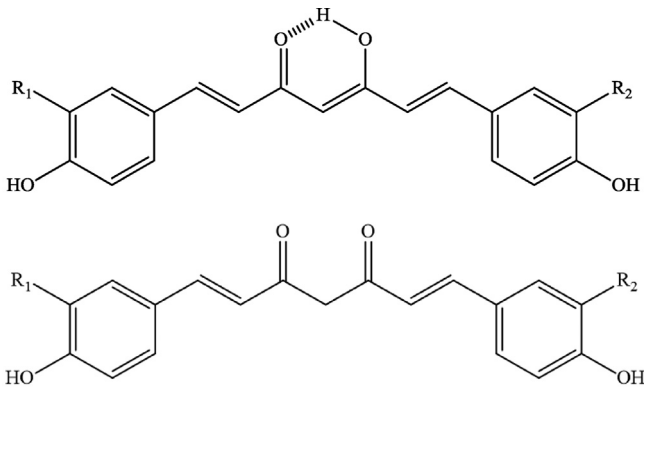

	R_1	R_2
Curcumin (CUR)	CH_3O	CH_3O
Demethoxycurcumin (DEM)	H	CH_3O
Bisdemethoxycurcumin (BIS)	H	H

Fig. 1. Cheto-enol (top) and bis-cheto (bottom) tautomers of curcuminoids.

surized liquid extraction [16] and supercritical fluid extraction [12,17,18]. However, according to the international authorities (FAO/WHO and European Commission) monitoring the development and commercialisation of food additives, a limited number of solvents is permitted in the preparation of curcuminoid-based products [19]. In particular, acetone, methanol, ethanol, isopropanol, hexane, ethyl acetate and supercritical carbon dioxide are the solvents accepted in the extraction of CUR and its analogues from turmeric.

Ethyl lactate (EL) is one of the most important bio-based solvents [20] regarded as a convenient alternative to petrochemical solvents in the perspective of developing sustainable industrial processes. EL can be produced from renewable raw materials, is completely biodegradable, non-corrosive and non-ozone-depleting. It is miscible with both water and hydrophobic liquids and exhibits excellent solvent properties comparable to those of petroleum-based solvents. Due to its low toxicity, use of EL in food and pharmaceutical products has been approved by both the US Food and Drug Administration and the European Union. Recent studies have demonstrated the great efficiency of EL in the extraction of nutraceuticals from various plant matrices [21–25].

In the present work, we investigated by high-performance liquid-chromatography (HPLC) the potentiality of EL as a solvent for the extraction of the curcuminoids from turmeric. In addition to pure EL, we evaluated the extraction efficiency of binary and ternary mixtures of EL with water and ethanol (EtOH). EtOH was selected among the organic solvents miscible with water because, apart from being tolerated in food industry and medicine, is known to exhibit a good ability in the solubilisation of curcuminoids [6]. To explore how changes in the proportions of the three solvents influence the extraction yield, we applied response surface methodology combined with a mixture experimental design [26]. In particular, an augmented simplex-centroid design was used to define compositions of binary and ternary mixtures to be considered together with pure liquids ensuring a quite homogeneous coverage of the composition domain. Finally, stability of CUR and its analogues in pure EL and EL-water mixtures was investigated.

2. Experimental

2.1. Samples, chemicals and solvents

Turmeric from India was purchased from the section of exotic foods of a local supermarket. Curcumin (E 100) food dye was obtained from a provider of food ingredients. Standards of CUR (purity 99%), DEM (purity 98%) and BIS (purity 98%) were obtained from Sigma Aldrich (Saint-Louis, MO, USA). EL (purity >98%) from Sigma Aldrich, HPLC-grade ethanol (Carlo Erba Reagenti, Milan, Italy) and double deionized water, obtained from a Milli-Q filtration/purification system (Millipore, Bedford, MA, USA), were used for the preparation of extraction mixtures. HPLC-grade acetonitrile (Sigma Aldrich) was used for the preparation of the HPLC mobile phases. Stock solutions of individual curcuminoids at concentration of 1 mg/mL were prepared in HPLC-grade methanol (Sigma Aldrich) and stored at 4 °C.

2.2. HPLC analysis

The chromatographic system consisted of two Model 510 pumps (Waters, Milford, MA, USA), a Pump Control Module II (Waters), a Model 7725i sample injector (Rheodyne, Cotati, CA, USA) equipped with a 20 μL loop and a Model 996 (Waters) diode array detector. Chromatographic data management was automated using a Millennium^{32} data acquisition system (Waters). All the analyses were performed on the analytical column Kinetex (Phenomenex, Torrance, CA, USA), 250 mm length by 4.6 mm i.d. and 5 μm particle size, connected to a 4.6 × 10 mm ODS2 Guard Cartridge (Waters) with 5 μm particle size. Concentration of the three curcuminoids was determined simultaneously by HPLC using a mobile phase composed of acetonitrile (ACN) and H_3PO_4 aqueous solution (0.1%) erogated at 1 mL/min under the application of linear gradient in which the ACN content grows from 45% to 80% (v/v) in 15 min. Some typical chromatograms collected under this condition are displayed in Fig. 2. Stability of curcuminoids in the circumstance of very fast degradation rate was investigated by analysing separately the solutions of individual compounds under isocratic elution conditions using an ACN-water 70/30 (v/v) mixture as mobile phase. Chromatograms were always recorded at a wavelength of 420 nm. Quantitative analysis was based on five-points calibration curves determined by analysing standard solutions in methanol of individual curcumoinoids in the concentration range 0.1–15 mg/L which provided R^2 values of 0.9999 for BIS, 0.9994 for DEM and 0.9974 for CUR.

2.3. Extraction of curcuminoids

50 mg of turmeric were transferred into a 50 mL volumetric flask successively filled with pure solvent or a mixture at the desired composition. The suspension, kept in the dark, was mechanically stirred for 30 min. After filtration, a 20 μL aliquot was analysed by HPLC under gradient elution conditions. Extraction of curcuminoids from curcumin (E 100) food dye was carried out according to the same procedure, but the extract was diluted 100 times before HPLC analysis. For the quantification of curcuminoids in turmeric or curcumin (E 100) food dye, extraction was carried with methanol under sonication for 60 min. This procedure, according to literature [27], ensures quantitative recovery of all the three curcuminoids and good precision. The limit of detection (LOD) and limit of quantification (LOQ) of the HPLC method were evaluated from the calibration curves as $3s_a/b$ and $10s_a/b$, respectively, where b is the slope and s_a the standard error of the intercept. The observed LOD and LOQ values were respectively 0.41 and 1.38 mg/L (BIS), 0.45 and 1.50 mg/L (DEM) and 0.58 and 1.94 mg/L (CUR). Six samples containing 1.5 mg/L of each curcuminoid were prepared by spiking