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Incorporation of 2-hydroxypropyl β-cyclodextrin in a biomolecule-based low-transition temperature mixture (LTTM) boosts efficiency of polyphenol extraction from *Moringa oleifera* Lam leaves

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ABSTRACT

A novel LTTM composed of glycerol and sodium acetate was used to assess the effect of 2-hydroxypropyl β-cyclodextrin (CD) on the effectiveness of polyphenol extraction from *M. oleifera* leaves (MoL). The extraction process was based on a 2^3-full factorial design and response surface methodology, to evaluate simultaneously the effect of CD concentration (C_{CD}), liquid-to-solid ratio ($R_{L/S}$) and temperature (T). By employing optimized conditions (C_{CD} = 1%, $R_{L/S}$ = 40 mL g^{-1}, T = 40 °C), the yield in total polyphenols (Y_{TP}) was 67.18 ± 5.04 mg gallic acid equivalents per g dry weight. This yield was significantly higher than that achieved with the extraction performed with LTTM alone or 80% aqueous ethanol. The extraction kinetics suggested that the extraction rate was slowed down in the presence of CD, without compromising the higher extraction capacity of the LTTM/CD. Extract characterization by means of liquid chromatography-mass spectrometry showed that there was no selective extraction of any particular polyphenol, indicating that CD acted only as an extraction booster.

1. Introduction

Moringa oleifera Lam (Moringaceae) is a plant widely distributed in many countries of the tropics and subtropics and it has a high nutritional value, containing bioactive substances, such as vitamins, β-carotene, aminoacids and various phenolics (chlorogenates and flavonol glycosides). *M. oleifera* has been claimed to possess a vast spectrum of medicinal properties, as various parts of this plant such as leaves, roots, flowers etc. may act as cardiac stimulants, antitumor agents, and may also have antiinflammatory, antihypertensive, antioxidant, antibacterial and antifungal activities (Anwar et al., 2007). *M. oleifera* leaves (MoL) in particular, have been shown to exhibit high antioxidant activity, ascribed to polyphenolic constituents (Shih et al., 2011; Sreelatha and Padma 2009). Conventional extraction methods to obtain polyphenol-enriched extracts with antioxidant activity employed aqueous ethanol (Vongsak et al., 2013), but alternative approaches including pressurised hot water extraction (Matshediso et al., 2015) and microwave-assisted extraction (Rodríguez-Pérez et al., 2016) have also been proposed.

To obtain a high-performance extraction of target compounds in a short process time, it is necessary to choose a selective solvent and most of the extraction techniques rely on the manipulation of the solvent polarity to increase solubility of the solute molecules. Besides its physical–chemical capacity in dissolving the target compound(s), the toxicity of a solvent to human beings and to the environment should also be considered. In this regard, low-transition temperature mixtures (LTTMs), also known as deep eutectic solvents (DES), comprised of low-cost, non-toxic and recyclable constituents made of natural substances

Abbreviations: AAE, ascorbic acid equivalents; CD, 2-hydroxypropyl β-cyclodextrin; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; GAE, gallic acid equivalents; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; LTTM, low-transition temperature mixture; MoL, *M. oleifera* leaves; RtE, rutin equivalents; TPTZ, 2,4,6-tripyridyl-*s*-triazine

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Nomenclature

A_{AR}	Antiradical activity (μmol DPPH g^{-1})
h	Initial extraction rate (mg g^{-1} min^{-1})
k	Second order extraction rate constant (g mg^{-1} min^{-1})
P_R	Reducing power (μmol AAE g^{-1})
$R_{L/S}$	Liquid to-solid ratio (mL g^{-1})
t	Time (min)
$t_{0.5}$	Time to achieve $Y_{TP(s)}/2$ (min)
t_R	Time to enter the regular regime (min)
T	Temperature (°C)
Y_{TFn}	Yield in total flavonoids (mg RtE g^{-1})
Y_{TP}	Yield in total polyphenols (mg GAE g^{-1})
$Y_{TP(s)}$	Yield in total polyphenols at saturation (mg GAE g^{-1})

(e.g. polyols, organic acids, amines etc.) may have an important prospect. By virtue of features including low vapour pressure, absence of flammability and water miscibility, LTTMs would appear as ideal solvents for a range of sustainable and eco-friendly applications (Paiva et al., 2014).

Cyclodextrins are natural cyclic oligosaccharides deriving from enzymic breakdown of starch, and they are composed of 6, 7 or 8 glucose residues linked by α(1 → 4) glycosidic bond. Cyclodextrin molecules possess a truncated cone shape, with a hydrophobic zone inside and a hydrophilic external surface, hence they are able to form inclusion complexes with sparingly water-soluble molecules (such as polyphenols), increasing their solubility (Pinho et al., 2014). In addition, cyclodextrin encapsulation of polyphenols contributes in higher stability and controlled release (Munin and Edwards-Lévy 2011). Recent studies suggested that combining 2-hydroxypropyl β-cyclodextrin (CD) with aqueous glycerol may effectively increase yield of polyphenol extraction (Kyriakidou et al., 2016; Mourtzinos et al., 2016). A similar outcome was reached for water extraction of apple flavonols, using various cyclodextrins (Parmar et al., 2015). In this framework, the investigation performed aimed at testing the efficiency of polyphenol extraction from MoL, using a combination of CD with a glycerol-based LTTM (Karageorgou et al., 2017). The process was first optimized deploying a central composite design and process evaluation was performed using kinetics, liquid chromatography-mass spectrometry characterization of the extracts obtained and representative antioxidant tests.

2. Materials and methods

2.1. Chemicals

2-Hydroxypropyl β-cyclodextrin (average MW 1460), gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), rutin (quercetin 3-*O*-rutinoside) and 2,4,6-tripyridyl-*s*-triazine (TPTZ) were from Sigma-Aldrich (Steinheim, Germany). HPLC grade solvents were used for all chromatographic analyses. Glycerol and aluminium chloride were from Fisher Scientific (New Jersey, U.S.A.). Sodium acetate dehydrate was from Penta (Prague, Czeck Republic). Folin-Ciocalteu reagent was from Fluka (Steinheim, Germany). Ferric chloride hexahydrate and ascorbic acid was from Acros Organics (Geel, Belgium).

2.2. Plant material and LTTM preparation

Detailed description of the collection and handling of *M. oleifera* leaves (MoL) has been presented elsewhere (Karageorgou et al., 2017). The material used for extraction was freeze-dried MoL powder, with an average mean particle diameter of 0.5 mm. LTTM preparation was carried out under optimised conditions (Karageorgou et al., 2017). Briefly, the LTTM was prepared using glycerol as the hydrogen bond donor (HBD) and sodium acetate as the hydrogen bond acceptor (HBA), at a molar ratio HBD:HBA of 6:1. Aqueous solution 80% (w/v) of this LTTM was employed for all experimentation.

2.3. Batch extraction process

Appropriate amount of MoL powder was mixed with the LTTM and CD in a 50-mL glass vial and extractions were performed under stirring at 600 rpm and 180 min resident time. The amount of MoL powder, CD concentration and extraction temperature were as dictated by the experimental design (Table 1). After the completion of the extraction, samples were centrifuged at 10,000 × *g* and the clear supernatant was used after diluted 1:20 with methanol.

2.4. Experimental design

A 2^3-full factorial design (Box-Behnken) was used (Paleologou et al., 2016), with the response being the yield in total polyphenols (Y_{TP}). The three independent variables taken into consideration were the CD concentration (C_{CD}) (X_1, varying between 1 and 9%, w/v), liquid-to-solid ratio ($R_{L/S}$) (X_2, varying between 10 and 40 mL g^{-1}) and temperature (T) (X_3, varying between 40 and 60 °C). The range chosen for each variable was based on previous results (Kyriakidou et al., 2016) and preliminary experiments. All three variables were coded at three levels, −1, 0 and 1 (Table 1), using the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad i = 1,\ 2,\ 3 \tag{1}$$

where x_i and X_i are the dimensionless and the actual value of the independent variable i, X_0 the actual value of the independent variable i at the central point, and ΔX_i the step change of X_i corresponding to a unit variation of the dimensionless value. The data obtained from the experimental design were analysed using regression analysis and least square regression methodology to determine the factors of the mathematical models. Analysis of variance (ANOVA) was used to evaluate the significance of the model. Three-dimensional graphs were constructed using the fitted model.

2.5. Extraction kinetics

Extractions were carried out as described above and samples were withdrawn at regular intervals, up to 180 min, to determine Y_{TP}. Control extractions with only LTTM (no CD addition) and 80% (v/v) aqueous ethanol were also performed, under identical conditions. Kinetics was performed using the double reciprocal model, as described

Table 1
Values and coded levels of the process (independent) variables used for the 2^3 full-factorial design.

Independent variables	Code units	Coded variable level		
		−1	0	1
C_{CD} (%, w/v)	X_1	1	5	9
$R_{L/S}$ (mL g^{-1})	X_2	10	25	40
T (°C)	X_3	40	50	60

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