Journal of Cleaner Production 154 (2017) 233–241

Contents lists available at ScienceDirect

Journal of Cleaner Production

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

Sustainable production of high purity curcuminoids from *Curcuma longa* by magnetic nanoparticles: A case study in Brazil

Maria Izabela Ferreira [a, 1], Massimiliano Magro [b, c, 1], Lin Chau Ming [d], Monica Bartira da Silva [d], Luan Fernando Ormond Sobreira Rodrigues [d], Débora Zanoni do Prado [a], Emanuela Bonaiuto [b], Davide Baratella [b], Jessica De Almeida Roger [b], Giuseppina Pace Pereira Lima [a], Monica Rossetto [e], Lucio Zennaro [e], Fabio Vianello [b, c, *]

[a] *Department of Chemistry and Biochemistry, São Paulo State University (UNESP), Institute of Biosciences, Botucatu, São Paulo, CP 510, 18.618-970, Brazil*
[b] *Department of Comparative Biomedicine and Food Science, University of Padua, Padua, Italy*
[c] *Regional Centre of Advanced Technologies and Materials, Department of Physical Chemistry and Experimental Physics, Faculty of Science, Palacky University, 17 Listopadu 1192/12, 771 46 Olomouc, Czech Republic*
[d] *Department of Horticulture São Paulo State University (UNESP), School of Agriculture, Botucatu, São Paulo, CP 510, 18.618-970, Brazil*
[e] *Department of Molecular Medicine, University of Padua, Padua, Italy*

A R T I C L E   I N F O

Article history:
Received 24 January 2017
Received in revised form
23 March 2017
Accepted 29 March 2017
Available online 31 March 2017

Keywords:
Turmeric
Curcumin production
Curcumin purification
Magnetic purification
Magnetic nanoparticles
Biomass reduction

A B S T R A C T

Large volumes of residual biomass could represent a matter of concern for large-scale purification of natural compounds, heavily influencing processing industries and logistic sizing, amount of solvents employed for the extraction processes and the final chemical and biological waste generation. In the present study, carried out in Brazil, the production of curcuminoids in *Curcuma longa* L. rhizomes was maximized as a function of plant maturity and solar UV exclusion. Noteworthy, curcuminoid content reached its maximum at around the end of the early vegetative phase (65 days after planting), henceforward plant growth determined only a detrimental accumulation of wastes. The harvesting at this early phase of plant maturation led to a more than tenfold reduction of exceeding biomass. In addition, by means of an innovative, sustainable and high efficient magnetic purification process for curcuminoids based on Surface Active Maghemite Nanoparticles (SAMNs), an outstanding yield of 90% and >98% purity, were achieved in a single magnetic purification step. The formation of the SAMN-curcuminoid complex (SAMN@curcuminoid) was demonstrated by optical and electron spin resonance spectroscopy and electron microscopy. The scalability of the purification method was proved by the application of an automatic modular pilot system for continuous magnetic purification of curcuminoids, capable of managing 100 L day^{-1} of SAMN@curcuminoid suspensions. The presented multidisciplinary approach embodies the fundamental principles of cleaner production providing a paradigm for the utilization of magnetic nanoparticles for biomolecule purification. Moreover, Brazilian agro-industries can benefit from sustainable innovation strategy outlined by the current study.

1. Introduction

The rational use of natural resources, the minimization of wastes as well as the sustainable product innovation are prerogatives of the research field on cleaner production. *Curcuma longa* (Zingiberaceae), also known as turmeric, is the most important source of curcuminoids, comprising curcumin and two related compounds, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) (Kulkarni et al., 2012). Nowadays, curcumin is used as a food supplement in several countries and the molecular basis for its pharmaceutical application has been already delineated for a wide range of diseases (Gupta et al., 2013). For this reason, curcumin and

* Corresponding author. Department of Comparative Biomedicine and Food Science, University of Padua, Agripolis - Viale dell'Università 16, Legnaro, 35020, PD, Italy.
E-mail address: fabio.vianello@unipd.it (F. Vianello).
[1] Authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jclepro.2017.03.218
0959-6526/

its derivatives are attracting an increasing interest in food and pharmaceutical field. Thus, the development of strategies for the improvement of pure curcuminoid production, from plant cultivation to molecule isolation, represents an important task. From this viewpoint, the influence of environmental factors and agronomic techniques on plant cropping is an important research topic, as these factors influence the accumulation of biomass as well as the biosynthesis of bioactive compounds.

In the present report, in the specific agro-climatic zone, under the effect of different light intensities and UV exclusion, the curcuminoid content in rhizomes reached its maximum at approximately the end of the early vegetative phase. Henceforward, the amount of curcuminoids did not significantly vary with plant growth. Thus, the anticipation of the harvest led to a significant waste minimization and a one order of magnitude lower biomass accumulation. Moreover, a sustainable and competitive purification procedure was developed in the present study. Pharmaceutical and food industries usually employ various chromatographic, ultrafiltration, or precipitation techniques or solvent extraction methods for isolating biomolecules of interest (Kitts and Weiler, 2003). These techniques show significant drawbacks when applied at the industrial scale, such as expensive instrumentation, time-consuming procedures, or large quantities of organic solvents and corresponding wastes. In particular, the main disadvantage of all standard column liquid chromatography procedures is the impossibility to cope with biological samples containing particulate material, so these techniques are not suitable for working at the early stages of the isolation/purification process, when suspended solids and fouling components are present in the sample (Turková, 1978). In this context, the use of magnetic nanoparticles can represent a valuable option as well as an innovation opportunity for cleaner production. Notwithstanding, most of the proposed syntheses of magnetic nanoparticle drastically limit their exploitation at an industrial level, as they are characterized by difficult scalability, impressive consumption of organic solvents, high costs and heavy impact on the environment. Furthermore, magnetic nanoparticles need in the most of the cases, to be stabilized to avoid self-aggregation and to guarantee long-term stability, pH and electrolyte tolerance, and proper surface chemistry. Coating processes are often cumbersome, time-consuming, and expensive, with low yields and, due to their lack of stability, coating tend to degrade. Coating deterioration represents a drawback as this phenomenon compromises the binding capability of the material. As a consequence, the use of nanomaterials as means for cleaner production could reasonably become a paradox, as they represent themselves sources of environmental hazard.

Recently, we developed a novel synthetic procedure for magnetic nanomaterial in the size range around 10 nm, constituted of stoichiometric maghemite (γ-Fe_2O_3) and showing peculiar surface chemical behavior, called surface active maghemite nanoparticles (SAMNs) (Magro et al., 2015a). Noteworthy, SAMNs applicability range from the biomedical field, as a long-term imaging nanoprobes (Cmiel et al., 2016; Skopalik et al., 2014), to the advanced material development, such as conductive DNA based metamaterials (Magro et al., 2015b) and electrocatalysis (Magro et al., 2016a), as well as in sensoristics (Urbanova et al., 2014) for the determination of glucose (Baratella et al., 2013), polyamines (Bonaiuto et al., 2016) and hydrogen peroxide (Magro et al., 2013, 2014a). SAMNs present a high average magnetic moment and high water stability as colloidal suspensions without any superficial modification or coating derivatization. Because of their unique physical and chemical properties, these naked iron oxide nanoparticles are currently used to immobilize various biomolecules, such as avidin (Magro et al., 2012a), curcumin (Magro et al., 2015c), citrinin (Magro et al., 2016b), rhodamine B isothiocyanate (Sinigaglia et al., 2012) and endogenous proteins from prokaryotes and eukaryotes (Magro et al., 2016c; Miotto et al., 2016; Venerando et al., 2013). Thus, SAMNs represent an ideal material for cleaner production as their synthesis is scalable and completely carried out in water. They do not need any kind of stabilizing coating, present a very high aqueous colloidal stability and, in contrast to their surface reactivity, they are structurally conserved upon binding to target molecules, such as curcumin (Magro et al., 2014b), DNA (Magro et al., 2015b) and chromate (Magro et al., 2016e).

In the present work, a purification method leading to the recovery of curcuminoids present in the mother biological matrix with high yield (90%) and purity (98%) was proposed. In addition, the feasibility of the proposed approach to be scaled up at industrial level was demonstrated by developing an automatic modular pilot plant, which was able of performing the continuous curcuminoid purification from the initial water-ethanol extract. The reported multidisciplinary approach, ranging from agronomy to nanotechnology and engineering, offers valuable insights for a sustainable production of pure curcuminoids at an industrial scale and for the economic valorization of Brazilian agro-industry.

2. Materials and methods

2.1. Materials

The cultivation experiments were carried out in an experimental farm of the Agronomic Science College, Universidade Estadual Paulista - UNESP, Botucatu - SP, in São Manuel - SP (22º46′0,571″ S and 48º34′11,32″ W, 744 m above sea level).

The experimental design was completely randomized with five light conditions and four harvest times, split plot in time, with five replicates consisting of six plants. Light levels were: A) UV exclusion; B) full sun, C) 30% shading; D) 50% shading and E) 70% shading. Harvest times were: 65, 128, 174, and 203 days after planting (DAP) corresponding to January, April, May, and June 2013.

The different light conditions were obtained by protected environments in tunnel structures, 3 m wide, 1.70 m high and 22.5 m long, with different coatings for light exposure control. The coating applied to exclude UV radiations was an anti-UV polyethylene film (150 μm) (Trifilme, Plastilux, Brazil), characterized by excluding more than 80% UV-B radiation. Coatings used to control shading levels were black polyethylene screens with 30%, 50% and 70% shading (Plastilux, Brazil).

An infrared gas analyser (LI-6400, Li-Cor Inc. Lincholn, NE, USA) was used to quantify photosynthetically active radiation (PAR). Measurements were carried out at 09:00 and 11:00 a.m. in cloudless days, every month during the plant growth. The mean PAR measured was used to define the amount of radiation for each light exposure condition: UV exclusion (610 $\mu mol\ m^{-2}s^{-1}$), 70% shading (360 $\mu mol\ m^{-2}s^{-1}$), 50% shading (500 $\mu mol\ m^{-2}s^{-1}$), 30% shading (630 $\mu mol\ m^{-2}s^{-1}$) and full sun (1200 $\mu mol\ m^{-2}s^{-1}$).

The soil was classified as Oxisol, sandy phase (Camargo et al., 1987; Santos et al., 2006) and exhibited the following chemical characteristics in the layer between 0 and 0.20 m: 12 g dm^{-3} organic matter; pH 5.4; 204 mg dm^{-3} P; 2.5 mmol dm^{-3} K; 39 mmol dm^{-3} Ca; 11 mmol dm^{-3} Mg; 71 mmol dm^{-3} CTC; $V = 76\%$.

Seed rhizomes, 12 cm long, were selected and homogenized. Planting was carried out on plots 0.4 m high, spaced 0.5 m apart, by placing each propagule 4 cm deep.

Necessary crop treatments, such as weed control and repairs in ridges were carried out during plant growth. Plots were irrigated daily, according to water demand recorded by tensiometers, thus soil was near its calculated field capacity. Irrigation was suspended 15 days before harvest.