Food Chemistry 203 (2016) 505–512

Contents lists available at ScienceDirect

Food Chemistry

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

Citrinin mycotoxin recognition and removal by naked magnetic nanoparticles

Massimiliano Magro [a,b], Denise Esteves Moritz [c], Emanuela Bonaiuto [a], Davide Baratella [a], Milo Terzo [a], Petr Jakubec [b], Ondřej Malina [b], Klára Čépe [b], Glaucia Maria Falcao de Aragao [c], Radek Zboril [b], Fabio Vianello [a,b,*]

[a] Department of Comparative Biomedicine and Food Science, University of Padua, Italy
[b] Regional Centre of Advanced Technologies and Materials, Department of Physical Chemistry, Palacký University in Olomouc, Czech Republic
[c] Department of Chemical and Food Engineering, Campus Reitor João David Ferreira, Federal University of Santa Catarina (UFSC), Florianópolis (SC), Brazil

ARTICLE INFO

Article history:
Received 17 September 2015
Received in revised form 12 December 2015
Accepted 30 January 2016
Available online 17 February 2016

Keywords:
Food safety
Monascus
Citrinin
Magnetic nanoparticles
Mycotoxin removal
Natural dyes

ABSTRACT

Citrinin is a nephrotoxic mycotoxin which can be synthesized by *Monascus* mold during the fermentation process in foods. *Monascus*, generally described as red mold, is a red-pigmented filamentous fungus attracting a great interest for the production of natural dyes and cholesterol-lowering statins. We individuated a specie of *Monascus* producing high amount of natural dyes. However, this high pigmentation was correlated with the production of citrinin. Peculiar magnetic nanoparticles, synthesized in-house and called "Surface Active Maghemite Nanoparticles" (SAMNs), are proposed as an efficient and reliable mean for citrinin removal from *Monascus* treated foods. The nanomaterial efficiency for citrinin binding was proved on *Monascus* suspensions, and SAMN@citrinin complex was characterized by Mössbauer spectroscopy and magnetization measurements, showing that SAMNs resulted structurally and magnetically well conserved after citrinin binding. SAMNs are excellent and stable magnetic nano-carrier for toxin removal, which can be applied in food industry.

1. Introduction

Currently about 70% of colorants employed in processed foods are ordinary chemical dyes. In the past decades natural dyes lost market, as synthetic chemicals have better consistency and stability, greater range of colors and lower cost. Notwithstanding, in recent years consumer concern pressed for increasing the use of natural dyes obtained from plants and microorganisms. Natural dyes are commonly used for foods, such as meat, sweets, fruit juices, etc., and in the pharmaceutical field, mainly in the encapsulation of active ingredients. In 2007, the market of natural dyes was estimated around 1.15 billion dollars and, according to Leatherhead Food International, since 2004, it has risen of about 2.5% per year (Mapari, Thrane, & Mey, 2010).

Fungi from the *Monascus* genus are a promising source for natural color additives. *Monascus*, also known as red mold, produces at least six different pigments (Meinicke et al., 2012), and more than 50 patents are available about dye production by *Monascus* (Hajjaj, François, Goma, & Blanc, 2012), indicating the great technological interest aroused about this fungus as a source of natural dyes. Furthermore, discoveries of cholesterol-lowering statins produced by *Monascus* have prompted research into its possible medical uses. Notwithstanding, depending on growing conditions, *Monascus* can produce a mycotoxin, citrinin ((*3R,4S*)-8-hydroxy-3,4,5-trimethyl-6-oxo-4,6-dihydro-*3H*-isochromene-7-carboxylic acid), which is also produced by several species of the genera *Aspergillus* and *Penicillium* (EFSA, 2012), with risks for public and animal health, related to its presence in food and feed. Generally, citrinin is formed in plants after harvest, and occurs mainly in stored grains, but also in other products, such as beans, fruits, herbs and spices, and also in spoiled dairy products (Da Lozzo, Mangrich, Rocha, De Oliveira, & Carnieri, 2002). Citrinin control and detection in foods appears very relevant for food safety, as citrinin is nephrotoxic, and may cause serious health problems (Xu, Jia, Gu, & Sung, 2006). This mycotoxin was correlated to a decrease in the cellular ATP content and to an increase of the generation of reactive oxygen species in cells (Da Lozzo, Oliveira, & Carnieri, 1998; Da Lozzo et al., 2002; Hoehler, Marquardt, McIntosh, & Xiao, 1996; Ribeiro, Campello, Chagas, & Kluppel, 1998; Ribeiro,

* Corresponding author at: 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).

http://dx.doi.org/10.1016/j.foodchem.2016.01.147
0308-8146/

Chagas, Campello, & Kluppel, 1997; Stormer & Hoiby, 1996). Thus, its removal from fermentation broths and foods is an attractive task for health, environmental and economic reasons (Da Lozzo et al., 2002).

Ideally, foods fermented by *Monascus* should involve the selection of strains producing large amount of bio-pigments, but, obviously, no citrinin.

Food industry is slowly accepting nanotechnologies, and this is not surprising as public preference for "natural" food products has historically inhibited the implementation of emerging technologies in food processing. Indeed, while public opinion about nanotechnology applications has ranged from neutral to slightly positive (Currall, King, Lane, Madera, & Turner, 2006; Satterfield, Kandlikar, Beaudrie, Conti, & Harthorn, 2009), recent studies suggested that consumers remain wary about "nanofoods" (International Risk Governance Council, 2009; Siegrist, Keller, Kastenholz, Frey, & Wiek, 2007). Nevertheless, scientists and industry stakeholders have already identified potential uses of nanotechnology in virtually every segment of food industry, from agriculture to food packaging, nutrient supplementation and, of course, food processing.

One of the main drawbacks drastically limiting the exploitation of nanoparticles at industrial level is attributable to the difficulty of moving from laboratory to large scale production. Often the synthesis of nanomaterials involves impressive consumption of solvents, high costs and heavy impact on the environment. Thus, to be really taken into consideration, nanoparticles should be produced by a protocol responding to specific requirements, such as cost effectiveness and environmental friendliness. In particular, generally, magnetic nanoparticles need to be stabilized to avoid aggregation and to guarantee long-term stability, pH and electrolyte tolerance, and proper surface chemistry. Nanoparticle coating processes are often cumbersome, time-consuming, and expensive, with low yields, limiting their massive application. By this point of view, novel magnetic nanoparticles, called SAMNs (Surface Active Maghemite Nanoparticles) represent an ideal material, as their synthetic protocol is suitable for being scaled up to an industrial level and is carried out in water, without the employment of any organic solvent (Magro, Valle, Russo, Nodari, & Vianello, 2012). SAMNs represent a new class of naked superparamagnetic maghemite nanoparticles, constituted of stoichiometric maghemite (γ-Fe_2O_3) in the dimension range around 10 nm (Magro et al., 2012). Moreover, SAMNs are stable in water for several months as colloidal suspensions without any superficial modification or coating derivatization, displaying the ability to selectively bind several biomolecules (Magro et al., 2014; Magro and Baratella et al., 2014; Magro et al., 2015; Sinigaglia et al., 2012; Venerando et al., 2013).

Chelating properties of citrinin toward iron(III) were already reported in literature (Da Lozzo et al., 2002). Thus, the availability of iron(III) atoms on the surface of SAMNs was exploited for the recognition and magnetically removal of citrinin from *Monascus* in biological matrixes. In the current report, we present a promising nanoparticle application for food safety, aimed at the removal of citrinin from *Monascus* suspensions.

2. Materials and methods

Chemicals were purchased at the highest commercially available purity and were used without further treatment. Citrinin, iron(III) chloride hexahydrate (97%), sodium borohydride ($NaBH_4$), tetramethylammonium hydroxide, perchloric acid, ammonia solution (35% in water) were form Sigma–Aldrich, Italy.

2.1. Instrumentation

Optical and fluorescence measurements were performed in 1 cm quartz cuvettes using a Cary 50 spectrophotometer and a Cary Eclipse fluorescence spectrometer (Varian Inc., Palo Alto, CA, USA), respectively.

Transmission electron microscope (TEM) images were acquired by a JEOL 2010 microscope (JEOL Ltd., Tokyo, Japan), operating at 200 kV with a point-to-point resolution of 1.9 Å. The ^{57}Fe zero-field Mössbauer spectra were recorded at 300 K, employing a MS2007 (RCPTM, Czech Republic) Mössbauer spectrometer (Pechousek, Jancik, Frydrych, Navarik, & Novak, 2012), operating in a constant acceleration mode and equipped with a 50 mCi ^{57}Co(Rh) source. The values of isomer shift were referred to the metallic iron (α-Fe) at room temperature. The acquired Mössbauer spectra were fitted by the Lorentzian line shapes using the least-square method in the MossWin software program (Klencsár, Kuzmann, & Vértes, 1996).

A superconducting quantum interference device (SQUID, MPMS XL-7, Quantum Design) was used for the magnetization measurements. The hysteresis loops were recorded at a temperature of 300 and 5 K and in externally magnetic fields ranging from −5 to +5 T. The zero-field-cooled (ZFC) and field-cooled (FC) magnetization curves were recorded in a sweep mode of 1.8 K min^{-1}. The ZFC curve was measured after cooling the sample from 300 to 5 K in a zero magnetic field and the measurement was carried out on warming from 5 to 300 K under the external magnetic field (0.1 T). In the case of FC curve, similar process was employed, but the sample was cooled in an external magnetic field (0.1 T).

A series of Nd–Fe–B magnets (N35, 263–287 kJ m^{-3} BH, 1170–1210 mT flux density by Powermagnet – Germany) was used for the magnetic driving of nanoparticles.

2.2. Microorganism cultures

Monascus spp. (native strain DDJ 012010) and *Monascus purpureus* (native albino strain DDJ 032008) were obtained from the culture collection of the Federal University of Santa Catarina (UFSC, SC, Brazil). *Monascus ruber* (strain CCT 3802) was obtained from the Tropical Culture Collection André Tosello (Campinas—SP, Brazil). Strain spore suspensions were frozen at −20 °C after adding 100 μL glycerol mL^{-1} as a cryoprotector and stored for 3 months.

The inoculum and cultures were prepared in rice medium (20 g L^{-1} rice, 5 g L^{-1} glycine and 20 g L^{-1} agar-agar), pH 5.5. *Monascus* was initially grown on rice medium in a Petri dish at 30 °C for 7 days and subsequently stored at 4 °C. A spore suspension was obtained by washing the Petri dish cultures with a sterile aqueous solution of 0.1% Tween 80 (Vendruscolo, Ribeiro, Espósito, & Ninow, 2009).

One milliliter of this spore containing solution was mixed with 1 mL of semisolid agar (0.2% w/v)), and this suspension was used for one-point inoculation on Petri dishes, containing rice medium (25 mL) or rice medium containing 1 g L^{-1} SAMN. Plates were incubated at 25 °C for 12 days. Radial growth of *Monascus* spp. on Petri dishes was measured by a caliper every 12 h and the growth was followed for 12 days.

2.3. Synthesis of surface active magnetic nanoparticles

A typical synthesis of nanoparticles was already described (Magro & Faralli et al., 2012; Magro et al., 2012; Magro and Sinigaglia et al., 2012) and can be summarized as follows: $FeCl_3 \cdot 6H_2O$ (10.0 g, 37 mmol) was dissolved in MilliQ grade water (800 mL) under vigorous stirring at room temperature. $NaBH_4$ solution (2 g, 53 mmol) in ammonia (3.5%, 100 mL, 4.86 mol mol^{-1} Fe) was quickly added to the mixture. Soon after the reduction