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Food grade nanostructured lipid carrier for cardamom essential oil: Preparation, characterization and antimicrobial activity

Fatemeh Keivani Nahr [a], Babak Ghanbarzadeh [a,*], Hamed Hamishehkar [b], Hossein Samadi Kafil [b]

[a] Department of Food Science and Technology, Faculty of Agriculture, University of Tabriz, P.O. Box 51666-16471, Tabriz, Iran
[b] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

Cardamom essential oil (CEO) loaded food grade nanostructured lipid carriers (NLCs) were produced using cocoa butter (as solid lipid) and Tween 80 (as surfactant) for application in aqueous-based foods. The results demonstrated that the developed NLCs had fine size (<150 nm) and high entrapment efficiency (>90%). Differential scanning calorimetry (DSC) results showed a less-ordered crystalline structure leading to high loading capacity. All samples exhibited perfect type crystal (or β-modification crystal) and a less crystalline state in X-ray diffractograms. Few changes were detected in the turbidity of systems after storage time, demonstrating that the NLCs did not show significant aggregation. Antimicrobial activity of CEO emulsion and CEO loaded NLC formulations were investigated by Broth Macrodilution method and confirmed that, encapsulation was able to protect the antimicrobial activity of CEO. The results showed that CEO-loaded NLC could be used as food supplements.

1. Introduction

In recent years, the tendency toward low fat foods has limited the use of lipophilic bioactive ingredients (e.g. carotenoids, omega-3 fatty acids, phytosterols and essential oils) in food industry. In this regard, food grade delivery systems may be used to include fat soluble nutraceuticals in aqueous-based foods and improve their bioavailability, functionality and physical and chemical stability during the processing and storage time (Babazadeh, Ghanbarzadeh, & Hamishehkar, 2016).

Cardamom, belonging to the family of *Zingiberaceae*, is obtained from the seeds of *Elettaria cardamomum Maton* and it is mostly cultivated in southern India, Sri Lanka, Tanzania and Guatemala (Morsy, 2015). The concentrated extract of cardamom spice, which is called cardamom essential oil (CEO), is used for flavoring a wide range of processed food, beverages, gelatin, confectionery and dairy products. The major flavor components of CEO are 1,8-Cineole, α-terpineol, α-pinene, β-pinene, limonene and allo-aromadendrene, of which volatiles limonene, a-terpinyl acetate, and 1,8-cineole impart most flavor identity to the CEO (Joshi et al., 2013; Mehyar, Al-Isamil, Al-Ghizzawi, & Holley, 2014; Sardar, Tarade, & Singhal, 2013). CEO exhibits anti-inflammatory, antifungal and antimicrobial activities (Mazumder, Kumria, & Pathak, 2014).

During storage, CEO would be chemically unstable when dispersed in an aqueous medium and in the presence of air, light, moisture, acid, and high temperatures and it will lose its flavor mainly by volatilization and oxidative degradation of terpinic and lipid components as well as other physical and chemical reactions. In this case, the oxidation products and intermediates may affect its sensory attributes (Krishnan, Bhosale, & Singhal, 2005; Mehyar et al., 2014; Najaf Najafi, Kadkhodaee, & Mortazavi, 2011). Therefore, there is a need for protection of CEO against adverse environmental factors.

In this regard, nano sized colloidal delivery systems seem to be suitable by preserving the included molecules from degradation, enhancing their poor solubility in water, bioavailability and nutritional value and also modulating their release (Lacatusu et al., 2013). On this point, nanostructured lipid carriers (NLCs) are the most promising systems because of high encapsulation efficiency, higher colloidal stability (due to the higher density of solid lipid), no need to the use of organic solvents in their production, less tendency to changes in particle shape and good physicochemical stability due to the less mobility of bioactive material in their solid matrix compared to the liquid matrix leading to minimum incidence of bioactive material expulsion during storage. NLCs are composed of a blend of solid and liquid lipids, which is surrounded by a surfactant. The presence of liquid oil into the centre of the

* Corresponding author.
E-mail addresses: Babakg1359@yahoo.com, Ghanbarzadeh@tabrizu.ac.ir (B. Ghanbarzadeh).

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solid lipid in NLC by creating imperfect crystals, provides larger empty spaces and higher solubility of bioactive material which in turn can potentially cause higher entrapment of bioactive material in the solid lipid (Fathi, Mozafari, & Mohebbi, 2012). Also, this less ordered structure minimizes the expulsion of bioactive material upon storage (Mitrea, Ott, & Meghea, 2014; Ni, Sun, Zhao, & Xia, 2015). There are several environment-compatible techniques to produce NLCs, including microemulsion technique, emulsification-solvent evaporation, emulsification-solvent diffusion method, high-pressure homogenization (Ni et al., 2015) and high shear homogenization. In order to produce NLC, at first, molten mixed lipids are emulsified and then the obtained dispersed phase is recrystallized (Esposito, Mariani, Drechsler, & Cortesi, 2015). In recent years, improvement of chemical stability and bioavailability of bioactive food components such as vitamin A (Pezeshki, Ghanbarzadeh, Mohammadi, Fathollahi, & Hamishehkar, 2014), quercetin (Ni et al., 2015; Sun et al., 2014), β-carotene (Qian, Decker, Xiao, & McClements, 2013; L. Zhang, Hayes, Chen, & Zhong, 2013) and hesperetin (Fathi, Varshosaz, Mohebbi, & Shahidi, 2013), etc. have been performed using NLC. Considering that, the use of natural remedies as food preservers is of high acceptability, the association of natural plant lipids (olive oil and cocoa butter) in the production of nanocarriers will provide cheap and renewable systems.

The main objective of the present work was to obtain CEO loaded NLC systems by the low energy emulsification method coupled with high shear homogenization and sonication. The physicochemical characteristics of developed NLCs including average particle size, zeta potential, loading capacity, thermal analysis, wide-angle X-ray diffraction and antimicrobial activity were investigated.

2. Material and methods

2.1. Material

Cardamom essential oil was provided by Zardband Co. (Iran). Cocoa butter was obtained from Shirin asal Co. (Tabriz, Iran) and extra virgin olive oil was purchased from Hojiblanca Co. (Spain). Other chemicals including Tween 80 and DPPH powder were analytical grade and were provided from Merck (Germany).

2.2. Preparation of NLC

The CEO loaded NLC was prepared by the low energy nanoemulsification method coupled with high shear homogenization and sonication. For this purpose, CEO was dissolved in olive oil and the mixture was added into melted solid lipid (cocoa butter). Then, the hot aqueous surfactant (Tween 80) solution was drop by drop added into the lipid phase under high shear homogenization (Silent crusher M, Heidolph, Nuremberg, Germany) at 20,000 rpm for 45 min. Then sonication was performed using an ultrasonic processor (Sonics, Vibracell, USA) for 10 cycle of 1 min ultrasonic processing with 1 min intervals. The ultrasonic processor was set at 70% of amplitude with 0.5 cycle per second (200 W, 24 kHz). The temperature of suspension was kept at 50 ± 5 °C during these processes. The produced hot oil in water nanoemulsion was cold down in the refrigerator (4 °C) for recrystallization of lipid phase, and finally the NLC was formed. In each formulation the weight ratio of cardamom essential oil to olive oil was varied as 1:1, 2:1, 4:1 and 1:0 in NLC1, NLC2, NLC3 and NLC4, respectively and the other factors were as the following: cocoa butter: 330 mg, Tween 80: 500 mg and distilled water: 25 ml.

2.3. Physicochemical characterization

2.3.1. Particle size and zeta potential

The mean particle size (z-average size), polydispersity Index (PDI) and zeta potential of CEO loaded NLCs were measured by Dynamic Light Scattering (DLS) and zetasizer (Malvern Instruments, U.K).

2.3.2. Loading parameters

The entrapment efficiency (EE%) and loading capacity (LC%) of CEO into NLC was determined by measuring the amount of both free and entrapped CEO in the dispersion medium. Free CEO was separated from encapsulated CEO using an ultrafiltration method (Millipore Amicon Ultra-15) with centrifugation. For this purpose, the samples were prepared by uniformly mixing of the NLCs with ethanol (50% w/w) (in the ratio of 1:7), followed by centrifugation for 5 min at 4000 rpm (Universal 320. UK.). The filtrate was collected, diluted with ethanol (50% w/w) and measured at λ_{max} = 235 nm by using UV–Vis Spectrophotometer (model Ultraspec 2000 Pharmacia Biotech. Canada). The amount of encapsulated CEO in NLC was determined by addition of 1 ml of chloroform to 1 ml of NLC (which was remained at the upper part of Amicon filter) and shaking for 15 min to collapse the carrier and free the encapsulated CEO. The chloroform phase contained free+ encapsulated CEO was measured spectrophotometrically at λ_{max} = 235 nm. The amount of CEO was calculated using the calibration curve in the concentration range of 0.0001–0.0006 mg/ml CEO, with a correlation coefficient of R^2 = 0.9896 (n = 6). The entrapment parameters were determined as Eqs. (1) and (2) (Babazadeh et al., 2016):

$$EE\% = \frac{Amount\ of\ encapsulated\ CEO}{Amount\ of\ encapsulated + free\ CEO} \times 100 \quad (1)$$

$$LC\% = \frac{Amount\ of\ encapsulated\ CEO}{Amount\ of\ total\ lipids - CEO} \times 100 \quad (2)$$

2.3.3. Scanning electron microscopy (SEM)

The surface morphology of obtained NLC was investigated by scanning electron microscopy (KYKY-EM3200). NLC sample was diluted 20 times with water. The sample was imaged using KYKY-EM3200 with an accelerating voltage of 26 kV.

2.3.4. Thermal analysis

Thermal analysis was performed by differential scanning calorimetry (DSC), using a DSC thermal analyzer (LINSEIS. DSC model P 10.). Samples were weighed (5 mg) in hermetically sealed aluminum pans. Operation conditions were heating to 80 °C at heating rate of 10 °C/min.

2.3.5. Wide-angle X-ray diffraction (XRD)

The overall crystalline phases of samples were determined by XRD measurement. XRD samples were prepared by freeze drying of NLC formulations. Radial scans of intensity were recorded at ambient condition over scattering 2θ angles from 10° to 60° (step size ¼ 0.05°, scanning rate ¼ 1 s/step), an operating voltage of 40 kV, and a filament current of 30 mA. The crystallinity index (ICr) of the samples was calculated from the ratio of the areas of the crystalline peaks to the total area under the scattering curve (Murthy & Minor, 1990). Crystal size was estimated using the Scherrer Equation:

$$D = \frac{k\lambda}{\beta \cos\theta} \quad (3)$$

where D is the crystal dimension (nm), k is the medium form factor which is considered as 0.9, λ is the wavelength of X-ray radiation