Powder Technology 313 (2017) 287–292

Contents lists available at ScienceDirect

Powder Technology

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

Design of microparticles containing natural antioxidants: Preparation, characterization and controlled release studies

Joana Aguiar [1], Raquel Costa [1], F. Rocha, B.N. Estevinho *, L. Santos

LEPABE, Departamento de Engenharia Química, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

ARTICLE INFO

Article history:
Received 18 December 2016
Received in revised form 26 February 2017
Accepted 3 March 2017
Available online 06 March 2017

Keywords:
Carboxymethyl cellulose
Microencapsulation
Natural antioxidants
Spray drying

ABSTRACT

Antioxidants are important compounds in the prevention of diseases like cancer, diabetes, cardiovascular, neurodegenerative diseases and premature aging, however they are very sensitive to the light, heat, oxygen and pH. In this study, caffeic acid (CAF), chlorogenic acid (CGA) and rosmarinic acid (RA), 3 natural antioxidants, were encapsulated by spray-drying using sodium carboxymethyl cellulose (Na-CMC) to overcome their limitations in industrial applications. Product yield values were around 40% and the encapsulation efficiency values were higher than 90%. Particles revealed a smooth spherical shape with mean diameter below 11 μm (considering volume size distribution). Microparticles loaded with CAF, CGA and RA were fully released after 45 min, 2 h and 4 h, respectively. Antioxidant activity was not compromised, highlighting the potential of spray-dried Na-CMC microparticles as carriers of natural antioxidants.

1. Introduction

According to recent studies, the excessive accumulation in the human body of products derived from oxygen and nitrogen reactions is responsible for premature aging and diseases like cancer, diabetes, cardiovascular and neurodegenerative diseases (Alzheimer and Parkinson) [1,2]. Antioxidants are a powerful tool to reduce the oxidative stress allowing the stopping or delay of a chain reaction through several mechanisms: reacting with free radicals, by depleting molecular oxygen, deactivating singlet oxygen, removing prooxidative metal ions, replenishing hydrogen to other antioxidants and absorbing UV light [3,4] and, furthermore, antioxidants can also be used as preservatives avoiding the oxidation of lipid ingredients [4].

Recently, interest in natural antioxidants is rising comparing to synthetic ones due to the consumer preference of organic and natural products with less additives and side effects [5,6]. However, natural antioxidants present some characteristics which may limit their industrial application such as sensitivity to heat, oxygen, pH and light [7,8] as well as unpleasant taste or smell, poor availability, high reactivity with other ingredients present in the product matrix and also high susceptibility to storage and processing conditions [9–11].

Microencapsulation may be used to overcome some of the restrictions of antioxidants and increase their applicability. Examples of final products where encapsulated antioxidants may be incorporated include cosmetic preparations [12], food products and supplements [13] and food packaging [14]. In the microencapsulation technique, solid, liquid or gaseous compounds are surrounded by a coating material creating a microparticle that can be used in food, pharmaceutical, cosmetic, agrochemical and textile industries [12,15,16]. Some advantages of microencapsulation techniques include higher stability by isolating active ingredients in order to prevent their deterioration, delayed evaporation in case of a volatile core, controlled and targeted release of active ingredients and improved product esthetics and marketing perception, while maintaining core properties [17–21]. Microencapsulation is also able to mask core undesired properties such as undesirable taste, odor or activity and to reduce the amount of ingredients in formulation being a cost saving alternative [22,23]. There are various microencapsulation techniques available, although, spray-drying is one of the most used, due its simplicity, relatively low cost, flexibility, high stability of the final dried product (due to low moisture content), high volume reduction, ease of handling, transportation and storage of the particles [11]. It also allows a continuous operation and appropriate encapsulation of many heat-labile (low-boiling point) materials due to the lower temperatures the core material reaches [9,24–26]. Currently, substances such as antibiotics, medical ingredients, additives, vitamins and polyphenols, among others are encapsulated in large-scale using spray-drying [9,23,25,27].

In this work, 3 natural antioxidants (caffeic acid (CAF), chlorogenic acid (CGA) and rosmarinic acid (RA)) were selected, considering their aforementioned advantages for industrial applications, and microencapsulated in sodium carboxymethyl cellulose (Na-CMC). CAF and CGA are polyphenols mainly found in the coffee tree although other sources include fruits (e.g. apple, pear, berries, plum), vegetables (e.g. sweet potato, lettuce, spinach), black teas, soy beans and wheat

* Corresponding author.
E-mail address: berta@fe.up.pt (B.N. Estevinho).
[1] Equal participation as first author.

http://dx.doi.org/10.1016/j.powtec.2017.03.013
0032-5910/

[28,29]. RA is also a polyphenol, found in a wide variety of plants from the *Lamiaceae* family: oregano, rosemary, marjoram, clary sage, thyme, basil [30]. According to literature, there are several benefits related to these compounds besides their strong antioxidant activity: anti-inflammatory, anti-microbial and anti-viral properties, as well as prevention of diseases associated with oxidative stress (namely cardiovascular, cancer and neurodegenerative) [30–38].

On the other hand, carboxymethyl cellulose (CMC) is a water soluble anionic cellulose derivative with carboxymethyl substitution groups and has applications in a wide range of fields such as food, cosmetic, pharmaceutical, textiles, and detergents. Carboxymethyl cellulose is already widely used in cosmetic and personal products as emulsifier, stabilizer, film-former and thickener agent [39]. Carboxymethyl cellulose hydrogel swelling behavior is pH and ionic strength dependent due to the presence of electrostatic charges in the polymer network, originated from sodium ion and the carboxymethyl group [40]. Films formed using carboxymethyl cellulose have in general a moderate strength in aqueous solutions although such property is dependent on the degree of substitution and molecular weight [41,42].

The main purpose of this work was to perform the microencapsulation by spray drying of these three different antioxidant compounds (CAF, CGA and RA) using Na-CMC, considering the advantages of these natural antioxidants and also of the encapsulating agent. After encapsulation, the microparticles were characterized in terms of shape and size distribution, and controlled release studies were performed. The antioxidant capacity of the encapsulated acids was also evaluated.

2. Materials and methods

2.1. Chemicals

Caffeic acid standard (Ref. C0625-2G) and chlorogenic acid standard (3-caffeoylquinic acid) were acquired from Sigma-Aldrich Chemical Co. (MO, USA). Rosmarinic acid (Ref. 536,954-5G) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol was obtained from VWR International (Fontenay-sous-Bois, France).

Carboxymethylcellulose sodium salt (viscosity 830 mPa·s (25 °C, 2% water)) was obtained from VWR International (Haasrode, Belgium) and methanol from VWR International (Fontenay-sous-Bois, France). ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) was purchased from AppliChem GmbH (Darmstadt, Germany), potassium persulfate was acquired from Panreac Química (Barcelona, Spain) and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Sigma-Aldrich Chemical Co. (MO, USA). Ethanol (96% purity) was obtained from AGA (Prior Velho, Portugal). Water was deionized in laboratory using Millipore™ water purification equipment (Massachusetts, USA). All the reagents used were of analytical grade purity.

2.2. Equipment

Weight measurements were performed with an analytical scale Mettler Toledo AG245 (Columbus, OH, USA). Quantification analysis of the antioxidants was accomplished using a spectrophotometer UV–Vis V-530 (Jasco), as well as the antioxidant activity assays. Microencapsulation was performed using a mini spray dryer BÜCHI B-290 (Flawil, Switzerland) with a standard 0.5 mm nozzle.

2.3. Validation of analytical method

CAF, CGA and RA were quantified by UV–Vis spectrometry (Jasco V-530 UV–Vis Spectrophotometer; Easton, USA) with detection at 313, 323 and 324 nm, respectively. Standard solutions of each antioxidant were analyzed in duplicate and three calibration curves were obtained. The main validation performance and reliability parameters of the analytical method for each antioxidant were determined.

The CAF calibration curve was linear from 0.25 to 15 mg/L and the linear regression was the following: Abs (AU) = 0.067 ± 0.001 [CAF] + 0.009 ± 0.006 (R^2 = 0.999). Values obtained for limit of detection (LOD) and limit of quantification (LOQ) were, respectively, 0.29 and 0.96 mg/L.

The CGA calibration curve was linear from 0.50 to 14 mg/L and the linear regression was the following: Abs (AU) = 0.0554 ± 0.0002 [CGA] − 0.003 ± 0.002 (R^2 = 1.000). Values obtained for LOD and LOQ were, respectively, 0.10 and 0.34 mg/L.

Finally, the RA calibration curve was linear from 1 to 15 mg/L and the linear regression was the following: Abs = 0.059 ± 0.003[RA] + 0.02 ± 0.03 (R^2 = 1.000). Values obtained for LOD and LOQ were, respectively, 0.50 and 1.66 mg/L.

The analytical method presented good intra-day and inter-day precision (coefficients of variation smaller than 5% except for the lowest concentrations) as well as great accuracy (recovery percentage close to 100%) for the antioxidants tested.

2.4. Preparation of microparticles by spray-drying

Microparticles were prepared by spray-drying as described by [43–45]. Separate solutions of Na-CMC (10 g/L) and antioxidants (10 g/L for CAF and CGA, and 1 g/L for RA) were prepared in ultrapure water, at room temperature, under stirring. Before encapsulation, Na-CMC and antioxidant solution were mixed together under stirring, during 30 min, at room temperature. The antioxidant concentration in the feed solution was around 2%. The experimental conditions were previously optimized [46,47]: feed flow rate of 4 mL/min (15%), inlet temperature of 115 °C, air pressure of 6.0 bar, 100% aspiration rate and nozzle cleaner set to 3. The outlet temperature was around 60 °C. The powders were collected and stored in falcon tubes, wrapped in aluminum foil, and stored at 4 °C.

2.5. Microparticles characterization

The size distribution of the microparticles was evaluated by laser granulometry using a Coulter Counter-LS 230 Particle Size Analyzer (Miami, FL, USA). For each experiment, a small sample of powder was suspended in ethanol before measurement. Samples were characterized by number and volume as an average of two runs of 60 s.

Particles morphology was assessed by SEM analysis using a High resolution (Schottky) Environmental Scanning Electron Microscope with X-Ray Microanalysis and Electron Backscattered Diffraction analysis: Quanta 400 FEG ESEM/EDAX Genesis X4M. Samples were coated with Au/Pd thin film for 100 s and with a 15 mA current, by sputtering, using the SPI Module Sputter Coater equipment.

2.6. Controlled release studies

The controlled release studies were performed in ultrapure water (pH 5.6) by weighing 3 mg of each antioxidant powder into separate flasks (in duplicate) and then adding 4.5 mL of water. The release was performed at room temperature (20–25 °C), under low stirring and in the absence of light (by wrapping the flask with aluminum foil). Samples were taken every 0, 1, 2, 5, 10, 20, 30, 45 min and 1, 2, 4, 6, 24 h to evaluate the amount of antioxidant released using the UV-spectrophotometry method.

2.7. Antioxidant activity

The antioxidant activity was estimated in duplicate using the ABTS radical scavenging assay, as described by [48]. Samples consisted of Na-CMC microparticles loaded with CAF, CGA and RA at maximum release (after 4 h in aqueous solution) and also non-loaded microparticles of Na-CMC. The antioxidant activity of samples containing CAF, CGA and RA in free solution with the same amount as in the microparticles was