Food Hydrocolloids 38 (2014) 56–65

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Preparation of alginate microspheres by emulsification/internal gelation to encapsulate cocoa polyphenols

Bryshila Lupo [a,b,*], Alicia Maestro [a], Montserrat Porras [a], José M. Gutiérrez [a], Carmen González [a]

[a] Group of Colloidals Systems Engineering, Department of Chemical Engineering, University of Barcelona, C/Martí i Franquès 1-11, 08028, Barcelona, Spain
[b] Universidad Centroccidental Lisandro Alvarado (UCLA), Program of Agro-industrial Engineering, Faculty of Agronomy, Núcleo Obelisco, Av/Florencio Jiménez, Barquisimeto, Venezuela

ARTICLE INFO

Article history:
Received 12 June 2013
Accepted 6 November 2013

Keywords:
W/o emulsion
Stability
Alginate microspheres
Internal gelation
Encapsulation
Cocoa polyphenols

ABSTRACT

Encapsulation of cocoa extract was performed by emulsification/internal gelation in alginate microspheres. A suitable gelling was determined with a minimum of 1.8×10^{-4} mol of Ca^{+2}/g alginate. The pH influence in alginate gels showed similar viscoelastic properties in the range of pH 3.5–10. Citrate and carbonate salts were uses as calcium sources, obtaining smaller spheres with citrate source. Moreover, SEM of microbeads made with citrate show uniform surface while the carbonate ones seem rough. Emulsions were formulated with several concentrations of Span 80, Span 85, Span 80-Tween 80 and polyglycerol polyricinoleate (PGPR). The most stable, also with the smallest microspheres were that prepared with PGPR. A shrinkage gelation factor of 0.8 was observed between drop size of emulsions and blank microspheres obtained, maintaining the shape and size distribution. When cocoa extract was encapsulated in microspheres, around 60% of retention was easily reached. The Peppas–Sahlin model fitted polyphenols release from microbeads, suggesting the existence of a relaxation/dissolution mechanism. The obtained cocoa microbeads could increase the daily intake of antioxidants when implemented in a food product.

1. Introduction

Antioxidants like vitamins, phenol, flavonoids and proanthocyanidins retard or inhibit oxidation of lipids and prevent certain diseases (Kris-Etherton et al., 2004; Owen et al., 2000; Woodside et al., 1999), such as cancer, artherosclerosis, neurodegenerative, inflammatory and cardiovascular diseases (Prior & Gu, 2005). Flavonoids are polyphenolic compounds widely distributed among plants mainly in seeds, fruits and beverages such as tea, wine and beer. The antioxidant activity of the phenolic compounds is mainly due to their redox properties, as they play an important role by neutralizing free radicals and oxidants (Feng Peng et al., 2003). Considering the above benefits and new ideas to reduce the use of commercial synthetic antioxidants, natural extracts rich in polyphenols arise as active ingredients that may be incorporated into food in order to make it functional. Because the addition of natural antioxidants may alter the color and flavor of foods, recent studies suggest the application of microencapsulation technique to mask these effects, to protect them from the environment or for controlling release into food (Champagne & Fustier, 2007; Deladino, Anbinder, Navarro, & Martino, 2008). For example, although cocoa is a rich source of polyphenols, the flavonoids contained offer an astringency and bitterness (Li et al., 2012) which could be masked by encapsulation.

The microencapsulation (ME) technique has been mainly described as a process in which small particles or droplets are surrounded by a homogeneous or heterogeneous coating, forming beads or capsules with various applications (Borgogna, Bellich, Zorzin, Lapsin & Cesáro, 2010). In this sense, microparticles, microcapsules or microspheres are defined as the product of the microencapsulation process depending on their morphology and internal structure (Anal & Singh, 2007). Basically, the microcapsules are differentiated of the microspheres by the distribution of active ingredient. In the first case, the active compound is included in solid or liquid form in the core of the bead. While, in the microspheres, the active ingredient is disperse and trapped in the matrix that forms the whole spheres (Zuidam & Shimoni, 2010). ME has an evident impact on the food industry. In food science and

* Corresponding author. Group of Colloidals Systems Engineering, Department of Chemical Engineering, University of Barcelona, C/Martí i Franquès 1-11, 08028, Barcelona, Spain. Tel.: +34 93 402 12 92, +34 93 402 12 88; fax: +34 93 402 12 91.
E-mail address: bryshilalupo@ucla.edu.ve (B. Lupo).

0268-005X/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodhyd.2013.11.003

biotechnology it involves the incorporation of ingredients like polyphenols, volatile additives, enzymes and probiotic bacteria in small capsules or spheres, giving them the chance to be stable, protected and preserved against nutritional and health loss and to eventually act as antimicrobial agents (Gouin, 2004; Nazzarro, Fratianni, Coppola, Sada & Orlando, 2009).

Alginate is one of the most widely used polymers in microencapsulation, as it forms a highly versatile, biocompatible and nontoxic matrix of gel that protects the active components of factors such as heat and moisture thereby enhancing stability and bioavailability (Funami et al., 2009). Moreover, alginate has many advantages both for human consumption and industrial application. Such aspects are compiled in the literature by Imeson (2010, Chap. 4) highlighting the prebiotic effect of the low molecular weight alginates, their benefits as daily fiber intake for the reduction of cholesterol levels in blood sugar and their ability to extend product shelf life (Goh, Heng, & Chan, 2012).

Microspheres of gelled alginate can be prepared by ionic gelation that may occur externally or internally. A source of Ca^{+2} is used in both cases. In external gelation the Ca^{+2} ions diffuse from an external source into the alginate solution at neutral pH. Contrarily, in internal gelation an insoluble calcium salt is already present inside the droplets before gelation occurs, and Ca^{+2} is released by acidification of the medium (Funami et al., 2009; Poncelet et al., 1995; Ribeiro, Neufeld, Arnaud, & Chaumeil, 1999; Ribeiro, Silva, Ferreira, & Veiga, 2005; Silva, Ribeiro, Figueiredo, Gonçalves, & Veiga, 2006). The most widely used encapsulation method is extrusion/external gelation, wherein the formation of beads containing the solution of alginate and the component to be encapsulated is accomplished by using an extruder device dripping on a bath of $CaCl_2$ solution which induces the gelation (Chan, Lee, Ravindra, & Poncelet, 2009). The main limitation of this technique is the large size of beads formed, which depends on the diameter of the nozzle extruder. Among other drawbacks, this method has the difficulty of large scale production because beads are formed one by one (Mofidi, Aghai-Maghadam & Sarbolouki, 2000). Further back in time, alginate encapsulation techniques has been applied for cells immobilization and drugs release due to the potential of methods and benefits of the hydrocolloid (Dornish, Aarnold & Skaudrud, 1996; Jankowski, Zielinska, & Wysakowska, 1997; Kearney, Upton, & Loughli, 1990; Wan, Heng, & Chan, 1992). Recent investigations have improved the extrusion technique by forming microbeads with electrostatic fields which has been even studied in previous investigation (Belščak-Cvitanović et al., 2011; Dhonal & Stepánek, 2010; Halle et al., 1994; Poncelet, Babak, Neufeld, Googen, & Burgaski, 1999; Yeh, Zhao, Lee, & Lin, 2009).

Emulsification/gelation technique is the process of dispersion of one liquid in another one, with alginate and active compound solved together in the dispersed phase. Therefore, when ionic induced gelation occurs, alginate forms the polymeric matrix trapping the active component inside. The addition of an emulsifier favors the formation and stability of the emulsion. Its nature and concentration influences the distribution of droplet size (Poncelet, 2001; de Vos, Faas, Spasojevic, & Sikkema, 2010). In this regard, the preparation of emulsion microspheres through the emulsification/gelation technique may be carried out by means of external or internal gelation. External gelation consists of emulsifying a watery solution of alginate-active component in a non-aqueous continuous phase, adding later to the medium a $CaCl_2$ solution to induce the gelation of droplets and promote separation of the formed microspheres. In emulsification/internal gelation an insoluble or partially soluble salt of calcium is already present inside the droplets of the water in oil emulsion (w/o) (Chan, Lee, & Heng, 2006; Gouin, 2004). An acid is then added to the medium that must diffuse along the continuous phase into the droplets. Then their pH decrease and Ca^{+2} ions are released, occurring gelation. Previous work in internal gelation mentions the difficulty of controlling the polydispersity of the spheres, with mean diameters between 50 and 1000 microns (Poncelet, 2001). In most of literature, the droplets of alginate solution contain $CaCO_3$ particles dispersed as the calcium source for internal gelation (Chen & Subirade, 2006, 2007; Chan, Lee, & Heng, 2002, 2006; Choi, Park, Hwang & Park, 2002; Funami et al., 2009; Guan, Chi, Yu, & Li, 2011; Silva et al., 2006; Zhao, Carvajal, Won, & Harris, 2007). Alternative calcium salts can be added in alginate-watery phase as Glucono-delta-lactone (GLG) (Amici, Tetradis-Meris, Pulido de Torres, & Jousse, 2008), tartrate, oxalate and citrate which have been preliminarily studied by Poncelet (2001) but without farther published investigation.

Surfactants like spans could be adequate as emulsifiers to formulate w/o emulsions as they are used in cakes, puddings and cosmetics, among others. Other emulsifiers with a low hydrophilic-lipophilic balance (HLB), like Polyglycerol polyricinoleate (PGPR) could be used. PGPR is well dissolved in corn and sunflower oil (Márquez, Medrano, Panizzolo, & Wagner, 2010; Márquez, Palazolo, & Wagner, 2007). It is also quite commonly used in chocolate manufacture, due to its excellent water-binding properties which inhibit the thickening of chocolate in the presence of undesired inclusions of water. According to FDA definition, PGPR is generally recognized as safe and so extensively used by the food industry (FDA, 2006; Gülserem & Corredig, 2012; Wilson, Van Shie, & Howes, 1998). Many investigations have examined the stability of w/o and multiple emulsions when PGPR is used (Gülserem & Corredig, 2012; Márquez et al., 2010; Su, Flanagan, Hemar, & Singh, 2006). However, according to our knowledge, only one published study uses this emulsifier to prepare alginate submicron beads and the authors use external gelation (Paques, van der Linden, van Rijn, & Sagis, 2013). Internal gelation, therefore, has not been suited with PGPR as emulsifier.

In the present study, alginate microspheres are prepared by emulsification/internal gelation with PGPR and several Spans as emulsifiers. Stability of emulsions and size distribution of resulting microspheres are studied and compared. The salts $CaCO_3$ and $Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$ (calcium citrate) are used as the calcium source at several concentrations. Moreover, it is evaluated the viability of cocoa polyphenol encapsulation through an experimental factorial design. Finally, the release profile of polyphenol from the prepared alginate microspheres is studied and fitted to a kinetic equation.

2. Materials & methods

2.1. Materials

Sodium alginate was supplied by Panreac. Sodium alginate was analyzed by H NMR spectroscopy using a Bruker DMX-500 (500 MHz) spectrometer. The ratio M/G was estimated to be 1.43 with monad frequencies fraction of guluronate ($F_G = 0.41$) and fraction of mannuronate ($F_M = 0.59$) units as well as diad and triad frequencies of $F_{GG} = 0.25$; $F_{MM} = 0.43$; $F_{GM} = 0.16$; $F_{GGG} = 0.13$; $F_{MGM} = 0.04$ and $F_{GGM} = 0.12$. The number average length of G-blocks was $N_{G>1} = 3.0$ (ASTM F 2259-03; Grasdalen, 1983; Santi, Coppetta, & Santoro, 2008). The weigh-average molecular weight (M_w) and the number-average molecular weight (M_n), $M_w = 1750$ kDa and $M_n = 668$ kDa, were determined by using a Waters 2695 Alliance model Gel Permeation Chromatograph. Water 2000-1000 Ultrahydrogel Columns were employed: $NaNO_3$ (0.1 M) was used as the eluting solvent. The universal calibration was constructed by using Dextrans standards with narrow-molecular-weight distribution (Ci et al., 1999; Sen, 2011). Calcium chloride and calcium citrate tetrahydrate at 96% and 99%