Food Hydrocolloids 49 (2015) 25–34

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Characterization of alginate beads with encapsulated cocoa extract to prepare functional food: Comparison of two gelation mechanisms

Bryshila Lupo [a, b, *], Alicia Maestro [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 17 October 2014
Received in revised form
12 February 2015
Accepted 17 February 2015
Available online 17 March 2015

Keywords:
Beads
External gelation
Internal gelation
Polyphenols release kinetics
Textural analysis

ABSTRACT

In this study we compared two gelation mechanisms for the preparation of alginate beads with encapsulated cocoa extract. We used the extrusion method to induce the external gelation [EG] or internal gelation [IG]. Beads with different cocoa and calcium concentrations were prepared by either gelation mechanism. Size, morphology and texture analysis showed clear differences in the structure of the beads. Those prepared by IG showed a more homogeneous and compact internal structure. Textural studies showed that EG beads were harder due to the more rigid shell formed when the calcium migrated from the exterior of the droplets. The cohesiveness also confirmed a significant difference between the strength of the internal structure of the two beads, in agreement with the morphology observed. At higher calcium concentrations, hardness increased and the diameter of the beads decreased in both cases. The release of polyphenols fitted the *Peppas-Sahlin* model, and release was delayed at higher calcium concentration, especially for the IG beads.

1. Introduction

The development of novel functional foods provides an opportunity to improve the quality of foods available to consumers and to benefit their health (Lopez-Rubio, Gavara, & Lagarón, 2006). Functional food may be prepared by incorporating capsules to deliver bioactive compounds (Wichchukit, Oztop, McCarthy, & McCarthy, 2013). The capsules are obtained by encapsulation techniques in order to protect the active compound from moisture and heat, and also to enhance stability and maintain properties as well as masking undesirable odors or tastes (Arshady, 1993; Fang & Bhandari, 2010; Gibbs, Kermasha, Alli & Mulligan, 1999; Patel & Velikov, 2011; de Vos, Faas, Spasojevic, & Sikkema, 2010). Hydrogels are used to encapsulate bioactive compounds due to their biosimilarity, aqueous interior and porous structure. Alginate is a nontoxic, versatile and economical hydrogel. The name generally refers to a family of polyanionic copolymers derived from marine kelp (Goh, Heng, & Chan, 2012; Hambleton, Debeaufort, Bonnotte, & Volleiy, 2009; Liu, Wang, Gao, Liu, & Tong, 2008). The most attractive property of alginate is the gel formation induced by the addition of various divalent cations (Draget, Skjåk-Bræk, & Smidsrød, 1997; Liu, Qian, Shu, & Tong, 2003). For alginate gelation, the encapsulation technique most widely used is the extrusion method, which consists of dropping an aqueous solution of polymer and active principle into a gelling bath of $CaCl_2$ (Chan, Lee, Ravindra, & Poncelet, 2009). This method can be used with a simple dripping tool like a pipette or syringe, as well as more complex tools (Zuidam & Nedovic, 2010). Another less common technique is emulsification/gelation, which involves the dispersion of one liquid in another, with alginate and active compound solved together in the dispersed phase. Thus, 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 (Poncelet, 2001; de Vos et al., 2010). The capsules or beads of gelled alginate can be prepared by ionic gelation, which 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. In contrast, in internal gelation an insoluble calcium salt is already present inside the droplets before gelation occurs, and Ca^{+2}

* 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).

http://dx.doi.org/10.1016/j.foodhyd.2015.02.023
0268-005X/

is released by acidification of the medium (Funami et al., 2009; Ribeiro, Silva, Ferreira, & Veiga, 2005; Silva, Ribeiro, Figueiredo, Gonçalves, & Veiga, 2006).

Cocoa extract is rich in polyphenols, and has been studied due to its antioxidant properties (Schinella et al., 2010). This natural extract is also used as an active ingredient, which can be incorporated into food in order to make it functional. However, this may alter the color and flavor of foods due the content of flavonoids, which produce astringency and bitterness. Encapsulation can mask these effects (Champagne & Fustier, 2007; Deladino, Anbinder, Navarro, & Martino, 2008; Li et al., 2012). Together with flavor, appearance and texture are the main sensory factors that determine food acceptability to consumers (Pons & Fiszman, 1996). Texture profile analysis (TPA), an instrumental method introduced by Szczesniak, Brandt, and Friedman (1963), has been used to describe the mechanical textural properties of foods, which can be calculated from the TPA curve obtained by a texturometer or similar instrument. The textural properties of alginate/cocoa beads are required for formulating bioactive capsules in order to identify their desirable characteristics for incorporation in food products (van den Berg, van Vliet, van der Linden, van Boekel, & van de Velde, 2007; Foo, Liong, & Easa, 2013). On the other hand, alginate as polyanionic polymer has been widely studied and applied as a delivery system of active compounds, due to the properties of its carboxyl groups as well as its biodegradability and absence of toxicity. Natural extracts rich in polyphenols arise as active ingredients that may be incorporated into food in order to make it functional by the application of encapsulation technique which protect actives compounds of environment, masks flavor (Belščak-Cvitanović et al., 2011) and controls their release into food, so that the study of the release profile of the polyphenols from alginate beads is an important way to better understand their kinetic behavior (Champagne & Fustier, 2007; Deladino et al., 2008).

In this study, we prepared alginate/cocoa beads by external and internal gelation mechanisms with the extrusion method. The influence of calcium and cocoa extract concentration and the type of gelation on the morphological characteristics, size and texture of beads was studied by statistical analysis. Our aim was to identify the most suitable formulation of alginate/cocoa beads as bioactive capsules to be incorporated in a food product. Additionally, we also performed kinetic studies of the release profile of the polyphenols (active principle) from the beads (polymeric matrix). For this purpose, we examined various delivery kinetics models to identify the best kinetic release profile of the active compound expressed as total polyphenol content over time.

2. Materials & methods

2.1. Materials

Sodium alginate was supplied by Panreac. Calcium chloride (96%) and calcium citrate tetrahydrate (99%), Folin-Ciocalteau reagent and Gallic acid were supplied by Aldrich. Glacial acetic acid and sodium hydroxide (6 M) were supplied by Panreac. Refined sunflower oil was supplied by Southern-Coosur oils; S.A. Neutral gelatin powder was purchased from Kraft Foods, Portugal. The cocoa extract with high polyphenol content was kindly provided by the Faculty of Pharmacy of the University of Barcelona (Spain). Deionized Milli-Q water was used in all experiments.

2.2. Preparation of alginate/cocoa beads

Two aqueous solutions with 2% w/w of sodium alginate and 1% or 3% w/w of cocoa extract as active principle were prepared and stirred until complete dissolution with an Ultra Turrax device (IKA-Werke GmbH & Co.KG, Germany) at 10,000 rpm, followed by sonication with an Ultrasonic homogenizer (Sonoplus Bendelin Electronic GmbH & Co.K.G, Model HD2070, Germany) equipped with titanium probe, 20 kHz frequency, 21 W power for 20 min. These solutions were stored for 24 h under refrigeration at 5 °C and protected from light, to ensure complete hydration and deaeration. Finally, when internal gelation was used the pH was adjusted to 6.7–6.9 by adding a solution of NaOH at 6 M under continuous stirring, using a pH/ISE measuring instrument (Schott® Instruments, SI Analytics GmbH, Model ProLab 3000, Germany), to avoid premature liberation of calcium ions due to the low natural pH of cocoa extract.

2.2.1. Beads prepared by external gelation

In order to prepare alginate/cocoa beads by external gelation with several proportions of cocoa extract and calcium, 20 ml of solution with alginate 2%/cocoa was dropped using a syringe without a needle for 5 min under manual control (50 ml BD Plastipak-2 mm internal diameter tube) into 80 ml of a second solution containing several proportions of $CaCl_2$, and stirred moderately. The beads formed immediately and were left in the original solution for 15 min to ensure internal gelification. The spheres were then filtered with a stainless steel strainer spoon prepared for spherifications, and washed in water and kept under refrigeration at 5 °C until performing the different analysis. Some beads were set aside for examination of their internal structure, others were measured for size, and others were used for analysis of their mechanical properties.

2.2.2. Beads prepared by internal gelation

In order to form alginate/cocoa beads by internal gelation with several proportions of cocoa extract and calcium, 20 ml of solution with alginate 2%/cocoa were mixed with a calcium citrate suspension until homogenization and dropped using a syringe without a needle for 5 min under manual control (50 ml BD Plastipak-2 mm internal diameter tube) into 40 ml of sunflower oil containing 80 μl of acetic acid previously solved to induce gelation. The beads formed were maintained in the gelling oil bath for 15 min to ensure internal gelification. These spheres were then treated and examined as described above. Table 1 shows all bead compositions.

2.3. Characterization of alginate/cocoa beads

2.3.1. Determination of size and morphology

Samples of 15 alginate/cocoa beads obtained from each formulation and type of gelation were taken at random and measured with a digital caliper (Topcraft GT-DC-02) in order to record their diameters. Data are presented as mean ± standard deviation (SD)

Table 1
Formulation of alginate/cocoa beads.

F_n	%Cocoa (w/w)	$CaCl_2$ (M)	Calcium citrate (M)	moles Ca^{+2}/g alginate ($.10^{-3}$)
E_1	1	0.002	–	0.4
E_2	1	0.005	–	1.0
E_3	3	0.002	–	0.4
E_4	3	0.005	–	1.0
I_1	1	–	0.05	0.4
I_2	1	–	0.25	1.0
I_3	3	–	0.05	0.4
I_4	3	–	0.25	1.0

Formulation, F_n; External gelation, E; Internal gelation, I; Subscript 1 and 2 for 1% w/w and 3 and 4 for 3% w/w of cocoa extract encapsulated; Also subscript 1 and 3 are beads formed with $0.4.10^{-3}$ mol Ca^{+2}/g alginate and 2 and 4 are beads formed with 1.10^{-3} mol Ca^{+2}/g alginate.