Anais da Academia Brasileira de Ciências
ISSN: 0001-3765
aabc@abc.org.br
Academia Brasileira de Ciências
Brasil

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Anais da Academia Brasileira de Ciências, vol. 89, núm. 3, julio-septiembre, 2017, pp. 1601-1613
Academia Brasileira de Ciências
Rio de Janeiro, Brasil

Anais da Academia Brasileira de Ciências (2017) 89(3): 1601-1613
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201720170071
www.scielo.br/aabc | www.fb.com/aabcjournal

Preparation and characterization of alginate and gelatin microcapsules containing *Lactobacillus rhamnosus*

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Manuscript received on January 31, 2017; accepted for publication on April 3, 2017

ABSTRACT

This paper describes the preparation and characterization of alginate beads coated with gelatin and containing *Lactobacillus rhamnosus*. Capsules were obtained by extrusion method using $CaCl_2$ as cross linker. An experimental design was performed using alginate and gelatin concentrations as the variables investigated, while the response variable was the concentration of viable cells. Beads were characterized in terms of size, morphology, scanning electron microscopy (SEM), moisture content, Fourier Transform Infrared Spectrometry (FTIR), thermal behavior and cell viability during storage. The results showed that the highest concentration of viable cells (4.2×10^9 CFU/g) was obtained for 1 % w/v of alginate and 0.1 % w/v of gelatin. Capsules were predominantly spherical with a rough surface, a narrow size distribution ranging from 1.53 to 1.90 mm and a moisture content of 97.70 ± 0.03 %. Furthermore, FTIR and thermogravimetric analysis indicated an interaction between alginate–gelatin. Cell concentration of alginate/gelatin microcapsules was 10^5 CFU/g after 4 months of storage at 8 ºC.

Key words: Probiotics, biopolymers, functional foods, microencapsulation.

INTRODUCTION

In recent years, consumer demands for foods that contribute directly to people's health have increased considerably. The current trend is that food is not only for nutritional purposes, but also to prevent nutrition-related diseases and improve the physical and mental well-being of consumers (Menrad 2003). In this regard, functional foods play a significant role.

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The term 'functional foods' was first introduced in Japan in the mid-1980s. Over the years, the concept has been modified in Japan, Europe, United States and in national and international organizations. A standard definition for functional food was established to facilitate greater communication between food experts and non-experts, scientists, government officials and the public. According to Martirosyan and Singh (2015) The Functional Food Center (FFC) announced a proposed new definition for 'functional food', and examples of these foods

include carotenids, dietary (functional and total) fiber, fatty acids, flavonoids, isothiocyanates, minerals, phenolic acids, plant stanols/sterols, polyols, phytoestrogens, soy protein, sulfides/thiols, vitamins, prebiotics and probiotics (Singh 2015).

According to FAO/WHO (2002), probiotics are live microorganisms (bacteria or yeasts), which when ingested or locally applied in sufficient numbers, confer one or more specified demonstrated health benefits for the host. Lactic acid bacteria (LAB) are the most studied bacteria within the probiotic field (Anal and Singh 2007). Among the LAB, *Lactobacillus rhamnosus* is typically associated with the human gastro-intestinal microbiota, and is one of the most widely studied probiotic bacteria. *Lact. rhamnosus* is a Gram-positive, non-motile, non-sporulating rod-shaped facultative anaerobic lactic acid bacterium. A number of health benefits have been claimed for *Lact. rhamnosus*, such as alleviation of inflammation or pathogen-induced barrier dysfunction (Lebeer et al. 2010, Donato et al. 2010), prevention of intestinal injuries induced by rotavirus diarrhea (Liu et al. 2013a), a decrease in the symptoms of atopic dermatitis in children aged 4-48 months (Wu et al. 2015) and prevention of gastrointestinal infectious (Hojsak et al. 2010).

According to the International Dairy Federation, the minimum concentration of probiotic should be around 10^6-10^7 CFU ml^{-1} at the end of product's shelf life (FAO/WHO 2002). In order to exert their health benefits, the minimum recommended therapeutic dose is 10^8–10^9 viable cells per day/dose (Hou et al. 2003). However, the viability of probiotic bacteria in functional foods is dependent on their survival during manufacture and storage and this is affected by several factors. Probiotics must survive in the acidic gastric environment and be able to reach the small intestine and colonize within the host (Corcoran et al. 2005), should be metabolically stable and active in the product (Gilliland 1989), besides factors such as presence of hydrogen peroxide, dissolved oxygen produced by starter cultures, high oxygen content and oxygen permeability through the packaging materials (Shah and Jelen 1990, Shah and Lankaputhra 1997). The oxygen permeability of the packaging material used currently for probiotic is not recommended due the oxygen toxicity in probiotic bacteria.

In order to improve the viability and stability of probiotics, different techniques have been proposed. The development of a microencapsulation matrix can provide a physical barrier during food processing, storage and in the conditions simulating of the gastrointestinal tract (D'Orazio et al. 2015). In this regard, microencapsulation of probiotics into hydrocolloid beads prepared by extrusion (Shaharuddin and Muhamad 2015) and emulsion (Zhao et al. 2017, Zheng et al. 2017) techniques or into spray-dried microparticles (Santos et al. 2014), has been investigated (Doleyres and Lacroix 2005). Alginate is often used as an encapsulating material because it has the benefits of being non-toxic and being readily available (Ding and Shah 2008, Chávarri et al. 2010, Pitigraisorn et al. 2017), besides this, it is a naturally occurring biocompatible and biodegradable linear anionic polysaccharide (Lotfipour et al. 2012).

Different types of *Lactobacillus* were encapsulated using an alginate hydrogel matrix as encapsulating agent, such as *Lactobacillus bulgaricus* (Meng-Yan et al. 2014), *Lact. plantarum* (Wang et al. 2016, Chen et al. 2012), *Lact. fermentum* (Martin et al. 2013) and *Lact. acidophilus* (Etchepare et al. 2016). Recent works have studied encapsulation of *Lact. rhamnosus* using whey protein and isomaltooligosaccharide (Liu et al. 2016), silica (Zhao et al. 2016), pectin (Li et al. 2016), chitosan-alginate (Gandomi et al. 2016), whey protein (Doherty et al. 2012) and alginate (Pirarat et al. 2015).

According to Mokarram et al. (2009), problems related to susceptibility to disintegration in the presence of excess monovalent ions, Ca^{2+} chelating agents, and harsh chemical environments

have been related with using alginate. Thus, coating the encapsulated beads with other polymers can improve their chemical and mechanical stability and the effectiveness of the encapsulation system (Krasaekoopt et al. 2006).

The objective of the present work was to optimize the conditions of microencapsulation of the probiotic strain *Lact. rhamnosus* ATCC 7469 using the extrusion method to obtain alginate/gelatin capsules and then to evaluate the survival of the encapsulated cells during storage at 8 ºC over a period of 4 months.

MATERIALS AND METHODS

MATERIALS

Lact. rhamnosus ATCC 7469 was obtained from the culture collection of the Antibiotics Department of the Federal University of Pernambuco, Brazil. Sodium alginate was purchased from Danisco (Denmark), pectin type LM was purchased from CP Kelco (USA), gelatin, $Na_2HPO_4.2H_2O$ and $KH_2PO_4.2H_2O$ were obtained from Vetec (Brazil), $Na_3C_6H_5O_7$, $CaCl_2$, NaCl and KCl were purchased from Quimica Moderna (Brazil), MRS broth was purchased from Merck (Germany), glucose was obtained from Nuclear (Brazil) and Agar was purchased from AgarGel (Brazil).

MICROORGANISM

Lact. rhamnosus was grown in De Man, Rogosa and Sharpe (MRS) broth at 37 ºC using 250 ml Erlenmeyer flasks containing 50 ml of medium. Cultivation was conducted under stationary conditions for 24 h. Culture was harvested by centrifugation (Excelsa II Centrifuge Mod. 206 BL) at 1,000 *g* for 10 minutes. The pellet was re-suspended in 30 ml of lyophilization medium (sucrose 10 % w/v and gelatin 1 % w/v) and the cell suspension was divided into 3 ml portions in vials. Samples were stored at - 80 ºC for 12 h and lyophilized for 24 hrs under vacuum (1,720 mT) at - 50 ºC. Culture was stored at 8 ºC and bacterial viability was determined using pour plate technique in MRS agar supplemented with 2 % (w/v) glucose in triplicate by cell resuspension in 3 ml saline solution (NaCl 0.85 % w/v).

CULTIVATION CONDITIONS

Lact. rhamnosus was cultured in MRS broth at 37 ºC for 24 hrs using 500 ml schott-flasks containing 100 ml of medium and an inoculum concentration of 5 % (v/v). For the preparation of inoculum, the content of one vial of lyophilized bacterium (10^7 CFU g^{-1}) was resuspended in 2 ml of 0.85 % w/v saline solution. Samples were withdrawn at various time intervals for analysis of concentration of viable cells with the objective of identifying the exponential phase of growth (data not shown), in order to obtain cells for encapsulation tests.

PREPARATION OF BEADS BY EXTRUSION

In order to define the conditions for *Lact. rhamnosus* microencapsulation and maximize the viability of the cells in the microcapsules, two independent variables (alginate concentration and gelatin concentration) were evaluated using a full 2^2 factorial design, with three central points (level 0), totaling seven experiments. The tests were performed randomly, and the data was analyzed using Statistica® 8.0 software (Statsoft, Tulsa, OK), with a 95 % confidence level. The experimental error was obtained from the mean and standard deviation of the central points. Table I shows the experimental conditions investigated for the experimental design:

Initially, cells were grown as described above for 14 hrs (late exponential phase). The cells were harvested by centrifugation at 3,500 rpm for 10 min, the pellets were washed once with 0.1M phosphate buffer saline (PBS) and resuspended in 5 ml of PBS so that the cell concentration in the final PBS/cell suspensions was approximately 10^9 CFU ml^{-1}.

TABLE I
Codified levels and actual values of the variables studied in the experimental design.

Assays	Sodium Alginate concentration (% w/v)	Gelatin concentration (% w/v)
1	1.0 (-1)	0.1 (-1)
2	5.0 (+1)	0.1 (-1)
3	1.0 (-1)	0.5 (+1)
4	5.0 (+1)	0.5 (+1)
5-7	3.0 (0)	0.3 (0)

The extrusion technique for the preparation of the beads, as described by Nualkaekul et al. (2013), was modified by altering the volume proportions of cell suspension and alginate. 5 ml of cell suspension was mixed with 5 ml of sodium alginate solution. The concentration of the sodium alginate solution varied according to Table I. Before use, polymer solutions were pasteurized by heating at 72 °C for 30 secs on a hot plate and then immediately put on ice to cool; it was subsequently used for coating. Pasteurisation was preferred to sterilisation at 121 °C, as it is conducted at a lower temperature and reduces the degree of polymer hydrolysis, which takes place during sterilization (Nualkaekul et al. 2013).

The cell/alginate mixture (1 ml) was extruded through a 0.8 mm diameter needle into sterile 0.15M $CaCl_2$ (20 ml) using a peristaltic pump (Gilson) at 1.6 ml min^{-1}. The beads were allowed to harden for 30 min and were then harvested using a sieve.

Coated beads were prepared by adding the beads produced previously into 30 ml of gelatin solution, according to experimental planning. Gelatin solutions were pasteurised as described above (72 °C, 30 s) and immediately put on ice to cool. The suspensions were mixed at 300 rpm for 10 min using an orbital shaker and the coated beads were harvested and washed with PBS. Beads were stored at 8 °C in penicillin tubes containing sterile water for further characterizations assays.

VIABLE CELL COUNTS

In order to count viable cells of *Lact. rhamnosus* in the beads, portions (0.2 g) of beads were solubilised in a solution of 1.5 % w/v sodium citrate and serially diluted in tubes containing sterile saline solution (NaCl, 0.85% w/v). 200 μL of the samples were plated on MRS agar supplemented with 2 % (w/v) glucose, using the pour plate technique. The plates were incubated at 37 °C for 48–72 h and the results of viable and cultivable cell counts were expressed by CFU/g.

BEADS CHARACTERIZATION

Beads obtained using the selected encapsulation conditions were characterized according to the methods described in the following sections.

Size

Direct observation of beads was carried out as follows: Twelve randomly selected beads were placed on dark paper and photographed with a digital photo camera (Canon EOS 70D) with a 100 mm macro lens. The scale bar was added from a calibrated digital image using the software ImageJ 1.47v.

Size measurements of beads were also evaluated under a microscope (Primostar, Zeiss) and photographed with a digital photo camera (Axiocam ERc5s, Zeiss). This procedure is robust since it works using the calibrated optical microscopy method for evaluation of the prepared microcapsules, as proposed by other authors (Abdelbary et al. 2012).

Micromorphological analysis

For micromorphological analysis, unused microscope slides were cleaned by soaking in 70 % alcohol for 10 minutes. Next, alginate beads were stained by 1 ml of 1 % aqueous toluidine blue (Sigma-Aldrich, St. Louis, MO) and rinsed

with distilled water for 30 minutes and 5 minutes, respectively. The stained capsules were placed on the glass slides, covered with a coverslip and photographed using a digital camera (Axiocam ERc5s, Zeiss) coupled to an optical microscope (Primostar, Zeiss), under oil immersion with x1000 magnification.

Scanning electron microscopy

The topographical properties of beads were investigated by scanning electron microscopy (SEM) (EVO LS15) at an accelerating voltage potential of 20 KV. Prior to examination, beads were prepared on aluminum stubs and coated with gold under an atmosphere of argon.

The moisture content

The moisture content of the beads (*Xp*) was determined according to the methodology described in agreement with the Association of Official Analytical Chemists (AOAC 2016). A 5 g portion of the beads was heated for 3 h at 105 ºC. After cooling in a desiccator, the beads were weighed and the procedure was repeated until a constant weight (*cw*) was obtained. The moisture content (*Xp%*) was calculated using Equation 1. The tests were performed in triplicate.

$$Xp(\%) = \left[\frac{5 - cw}{5}\right] x100 \quad (1)$$

FTIR and thermogravimetric analysis

Fourier Transform Infrared (FTIR) spectra were recorded with a spectrometer (Varian model 640-IR) using KBr discs and collecting data from 400–4000 cm^{-1}.

Thermal analysis was performed by differential thermal analysis (DTA) and Thermogravimetry Analysis (TGA) using a thermoanalyzer (Shimadzu 60WS, Tokyo, Japan). All measurements employed a linear heating rate of 10 ºC min^{-1}, nitrogen as carrier gas and a platinum empty pan as reference material. Portions of sample (10 mg) were heated linearly from 30 ºC to 800 ºC. The analyses were carried out in the Laboratory of Physics and Chemistry of Polymeric Materials at the Federal Rural University of Pernambuco.

Viability of encapsulated Lact. rhamnosus during storage

Beads of *Lact. rhamnosus* were stored in penicillin tubes containing sterile distilled water that were hermetically sealed and stored at 8 ºC. Probiotic survival was evaluated by performing viable cell counts immediately after the beads' preparation and after 30, 60, 90 and 120 days of storage.

RESULTS

THE EFFECT OF DIFFERENT BEAD FORMULATIONS ON THE VIABILITY OF *Lact. rhamnosus*

Figure 1 (a and b) shows the response surface and the contour curve, respectively, for the concentration of viable cells for experimental design as a function of the alginate and gelatin concentrations. The highest concentration of viable cells (4.2 x 10^9 CFU g^{-1}) was obtained for 1 % w/v of alginate and 0.1 % w/v of gelatin. The Pareto graph (Figure 1c) revealed that the alginate showed a statistically significant negative effect (- 4.59), indicating that higher viable cell counts were obtained at lower alginate concentrations. The variables of gelatin concentration and the factor of interaction between alginate/gelatin were not statistically significant, suggesting that the coating of the capsules with gelatin did not exert a positive effect on the cell viability of beads.

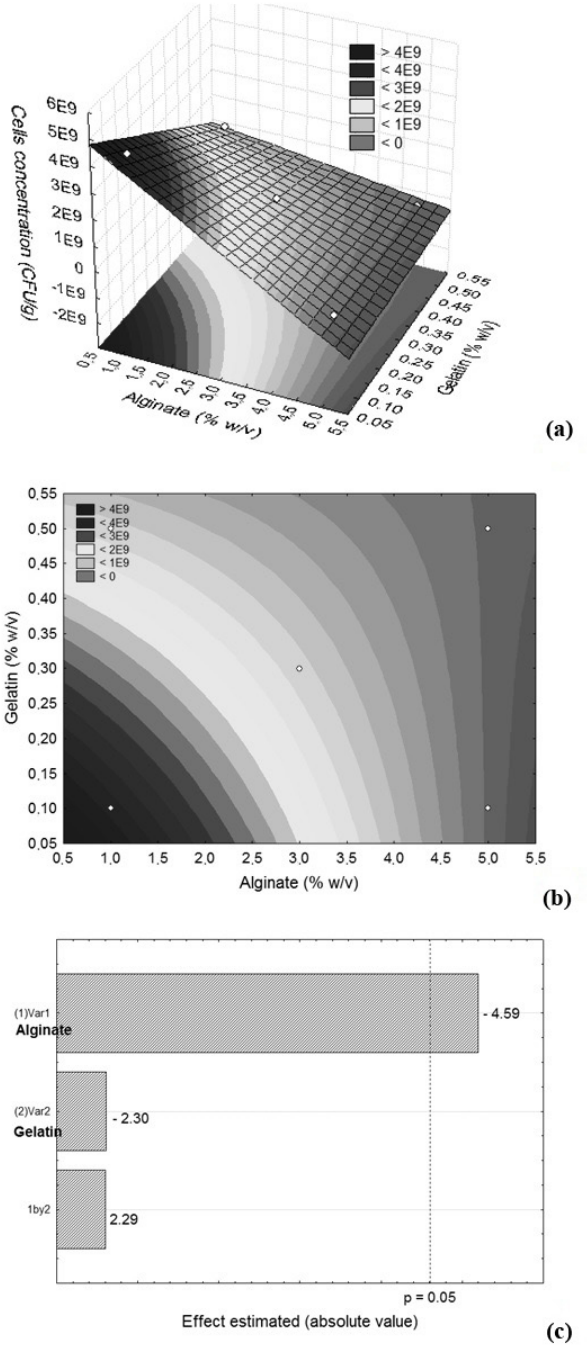

Figure 1 - Response surface **(a)**, contour curvature **(b)** and Pareto diagram **(c)** of viable cell concentration of *Lact. rhamnosus* as a function of the alginate and gelatin concentrations.

CHARACTERIZATION OF PREPARED BEADS: SIZE, MICROMORPHOLOGICAL ANALYSIS AND SCANNING ELECTRON MICROSCOPY

The alginate/gelatin beads for the selected formulation (1 % w/v of alginate and 0.1 % w/v of gelatin) were regular and spherical in shape and a white, opaque color (Figures 2a and 2b) and ranged in size from 1.53 to 1.90 mm. A cross section of bead (x1000 magnification) shows the integrity of *Lact. rhamnosus* cells entrapped within the capsule (Figure 2c). The presence of cells confirms that this technique is effective for microencapsulating this *Lactobacillus*.

Scanning electron microscopy images for capsules prepared using 1 % w/v of alginate and 0.1 % w/v of gelatin show that most of the capsules were predominantly spherical, although some were found to be elongated or irregular (Figure 3a) and exhibited a rough surface, not homogeneous and were constituted of polyhedral particles (Figure 3b). These characteristics are compatible with shrinkage occurring during the drying process (Sriamornsak et al. 2007).

THE MOISTURE CONTENT

The moisture content can be defined as the percentage weight of water in relation to the dry weight and is an important factor in evaluating the viability of microorganisms. The alginate/gelatin microcapsules containing *Lact. rhamnosus* presented a moisture content of 97.70 ± 0.03 %. Similar results were found by Belščak-Cvitanović et al. (2015), being characteristic of microparticles produced from polysaccharides that form gels and have the capacity to retain water.

FOURIER TRANSFORM INFRARED SPECTROMETRY (FTIR) AND THERMAL ANALYSIS

Figure 4 shows the IR spectra of sodium alginate (4a) and humid (4b) and dehydrated (4c) alginate/gelatin microcapsules. Thermal stability of

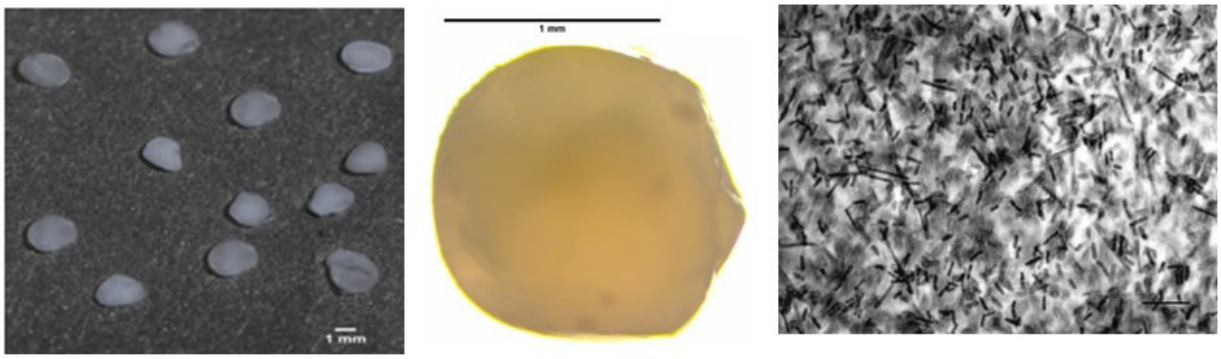

Figure 2 - Photographic images of alginate/gelatin beads (1 % w/v of alginate and 0.1 % w/v of gelatin). **(a)** Direct observation; **(b)** Microscopy image (x40 magnification); **(c)** Cross section of bead (x1000 magnification).

alginate/gelatin microcapsules was determined from Thermogravimetry Analysis (TGA) and Differential Thermal Analysis (DTA), as shown in Figure 5.

EVALUATION OF THE VIABILITY OF ENCAPSULATED BACTERIA DURING STORAGE

In this study, we tested the cell viability of alginate/gelatin microcapsules containing *Lact. rhamnosus* for the long-term storage stability at 8 °C for 120 days (Figure 6). We found that within four months of storage, viability was reduced from 10^9 to 10^5 CFU g^{-1}, which indicated around 5 log reductions in the bacterial population.

DISCUSSION

The results indicated that the concentration of viable cells decreases with an increase in the concentrations of the polymers. According to Chandramouli et al. (2004), alginate concentrations less than 1% (w/v) are quite difficult to encapsulate because of the decreased viscosity of the alginate solution and concomitantly less ion sites for the cross-linking. Also, concentrations more than 2 % (w/v) are too viscous and cause difficulties in the process of encapsulation. Moreover, the majority of previous reports in literature suggest that sodium alginate has generally been used at a concentration varying from 0.75 % to 2 % (w/v).

The beads characteristics are similar to those observed by Lotfipour et al. (2012), who observed results for diameters of alginate beads containing *Lact. acidophilus* ranging from 1.59 to 1.67 mm.

According to Totosaus et al. (2013), alginate microparticles usually had a core due to the heterogeneous gelation mechanism. This effect was reported by Skjak-Brak et al. (1989), who described that during the mechanism of gelification, the polymer concentration is much higher on the surface than in the centre of the gels, which results in uneven surfaces.

Sodium alginate (curve 4a) presented a band centered at 3430 cm^{-1} that corresponds to the functional group O-H. The band at 2929 cm^{-1} is attributed to the symmetrical and asymmetrical stretching of C-H. The peak observed at 2150 cm^{-1} is attributed to the group CO_2 group. The increase in the peak intensity at 1611 cm^{-1} corresponds the symmetrical stretching of COO^-. Peaks observed at 1416 cm^{-1} and 1304 cm^{-1} are attributed to the asymmetrical stretching of the COO^- and C-O group. Peaks at 1093 cm^{-1} and 1031 cm^{-1} are associated with the stretching with regards to the C-O group. Besides, peaks at 946 cm^{-1}, 890 cm^{-1} and 818 cm^{-1} are attributed to the C-H vibration of the pyranose (ring of the alginate) group (Falkeborg et al. 2015, Bekhit et al. 2016).

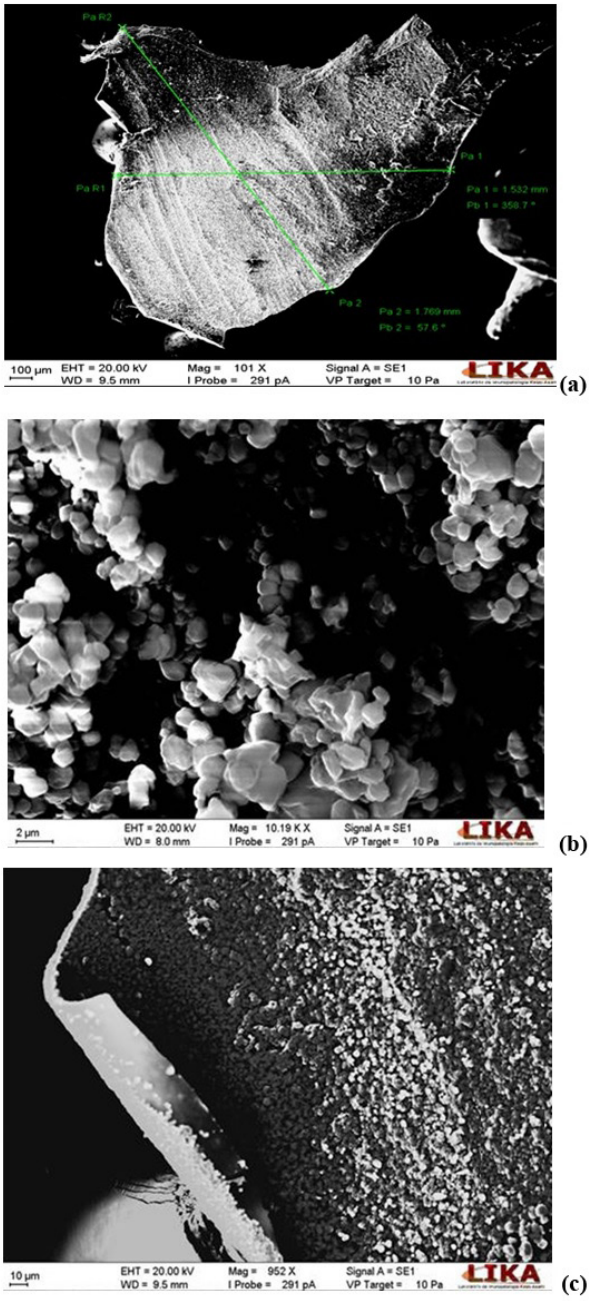

Figure 3 - SEM pictures of alginate/gelatin beads prepared with 1 % w/v of alginate and 0.1 % w/v of gelatin. **(a)** External aspect of the bead. Magnification of x101; **(b)** and **(c)** Internal aspect of the bead. Magnification of x10.19 K and x952, respectively.

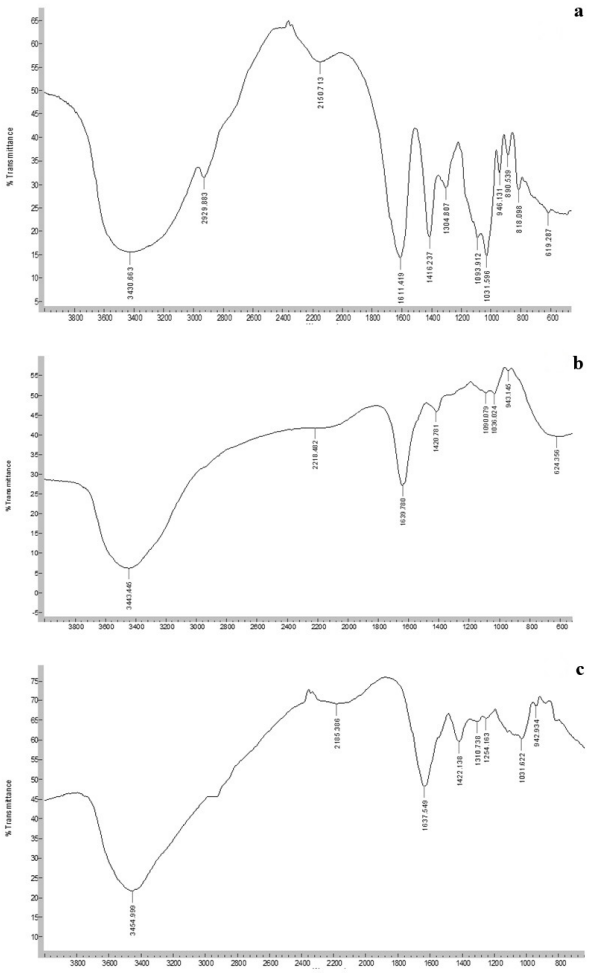

Figure 4 - FTIR spectra of sodium alginate (curve **a**), alginate/gelatin microcapsules humid (curve **b**) and dehydrated (curve **c**).

FTIR spectrum of alginate/gelatin microcapsules humid (curve 4b) showed a peak at 3443 cm^{-1} (O-H vibration) due to the presence of the great amount of water. A broad band centered around 2218 cm^{-1} is attributed to the CO_2 group. The increase in peak intensity at 1639 cm^{-1} is characteristic of the $–CONH_2$ group and indicates that the negative group of the alginate could be associated with the positive load of the gelatin. The gelatin, an amphoteric polymer that presents pH below his point isoelectric, could form a complex with anionic polysaccharides, such as the alginate, through electrostatic interactions (Saravanan and Rao 2010), leading to the formation

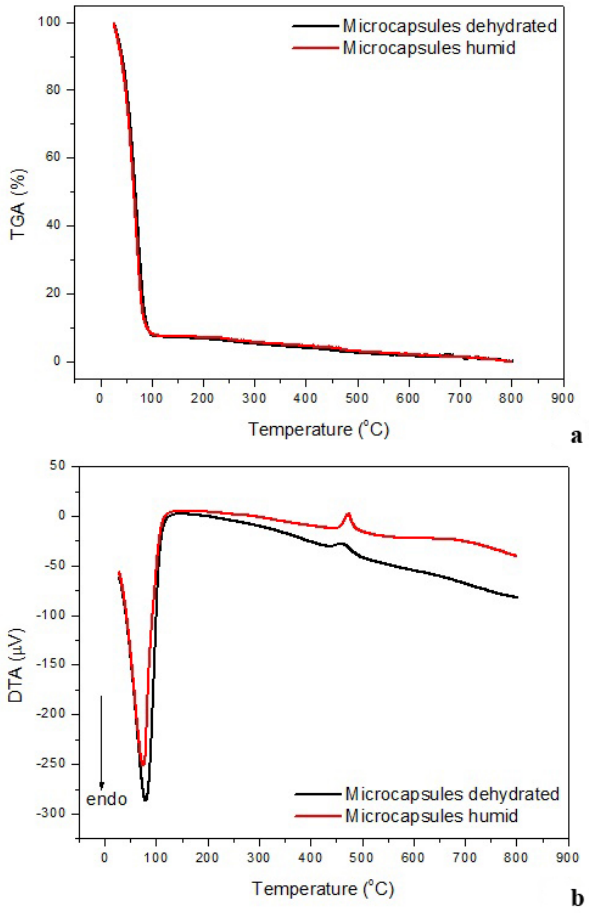

Figure 5 - TGA **(a)** and DTA **(b)** curves obtained for alginate/gelatin microcapsules humid and dehydrated.

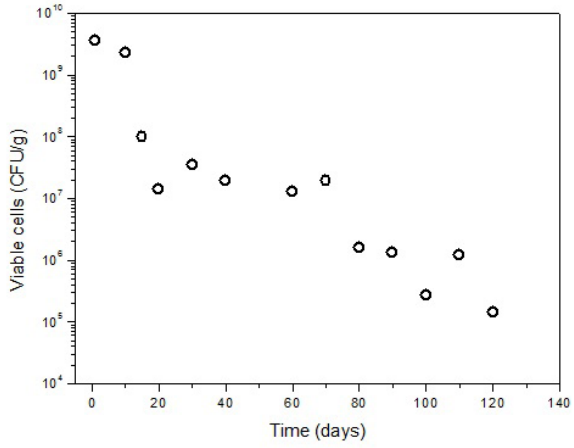

Figure 6 - Concentration of viable cells of *Lact. rhamnosus* in alginate capsules covered with gelatin for 120 days of storage at 8 °C.

of a polyelectrolyte complex, as described by Nualkaekul et al. (2013).

The peak at 1420 cm^{-1} is attributed to the asymmetrical stretching of COO-. Peaks at 1090 cm^{-1} and 1036 cm^{-1} are associated to the C-H group of guluronic units, and the peak at 943 cm^{-1} corresponds to the C-H group of the ring pyranose (El-Ghaffara et al. 2012, Xiao et al. 2014).

Alginate/gelatin microcapsules dehydrated (curve 4c) showed a peak at 3454 cm^{-1} regarding the functional group O-H linked to the hydrogen. The peak observed at 2185 cm^{-1} is attributed to the CO_2 group. The increase in the peak intensity at 1637 cm^{-1} corresponding to $CONH_2$ (C=O) indicates that the negative group of the alginate could be associated with the positive load of the gelatin. The peak at 1422 cm^{1} is due to the asymmetrical stretching of the COO- linked to the hydrogen of the alginate. Signals around 1310 cm^{-1}, 1254 cm^{-1}/1031 cm^{-1} and 942 cm^{-1} were attributed to vibrations of the C-N group of the gelatin, C-O and C-H groups, respectively. With the drying of the capsules, the molecules of water were affected and the bands decreased (Devi and Kakat 2013).

Comparing the FTIR spectrum profile of pure alginate with the microcapsules spectrum it can be observed that the FTIR spectrum of pure alginate showed a peak at 2929 cm^{-1} characteristic of C-H stretching, while FTIR spectrum of microcapsules presented group C-H associated to peaks 1090 cm^{-1} and 1036 cm^{-1} corresponding to guluronic units for microcapsules humid and a peak at 942 cm^{-1} for microcapsules dehydrated. In addition, the peaks at 1639 cm^{-1}/1637 cm^{-1} for microcapsules humid and dehydrated, respectively, are characteristic of the –$CONH_2$ group and indicate the interaction between alginate and gelatin.

TGA curves (5a) are typical of weight loss, and the major mass loss refers to a thermal event characteristically endothermic (between 30 °C and 80 °C – curve 5b). As this event begins at room temperature, it is assumed that this mass loss is

related to moisture. The mass loss of the dehydrated capsules is due to residual moisture, and the overlapping curves suggest that even with different initial contents of moisture this phenomenon occurs in the same way for both samples. The end of the thermal event occurs at a temperature above 100 °C, and this event may be associated with a bound water molecule. Therefore, by analyzing the results, sodium alginate has two types of water in its structure, a portion of unbound water related to moisture and another portion of water bound to the polymer.

The results indicate that up to 100 ºC the samples contained a low relative tenor of the organic matter, and starting from this temperature the samples degraded quickly during the heating up to 800 ºC.

Alginate/gelatin microcapsules showed an exothermic decomposition peak at 450 °C, a temperature higher than that observed by Sarmento et al. (2006) and Anbinder et al. (2011), who observed exothermic peaks for sodium alginate at 240 and 247 °C, respectively. This is probably due the presence of gelatin in the composition of microcapsules in this work, demonstrating that the presence of gelatin improve the chemical stability of the microcapsules. The oxidative degradation of the polymers can supply important information about behavior of polymeric materials under different atmospheric conditions (Liu et al. 2013b).

According to Stojanovic et al. (2012), the thermal decomposition of polymers includes the steps of dehydration and depolymerization accompanied by the rupture of C-O and C-C bonds and formation of CO, CO_2 and H_2O. Decomposition could be due to the combustion or even crystallization of the amorphous material (Gooch 2011).

Since that during the processing and/or storage of probiotics, cellular damage and loss of cell viability could occur, an appropriate microencapsulation assures that the microorganisms survive processing and remain viable throughout storage (Thomas et al. 2014). In this work, beads maintained the cell survival of *Lact. rhamnosus*, reaching 10^5 CFU g^{-1} after 4 months. On the contrary, when Trabelsi et al. (2014) studied the efficiency of immobilizing *Lactobacillus plantarum* TN9 strain in alginate using gelatin as a coating material, verified that the viability of encapsulated *Lact. plantarum* could be preserved more than 5.8 log CFU ml^{-1} after 35 days of incubation at 4 °C, and no effect was observed when gelatin was used. Chávarri et al. (2010) microencapsulated *Lactobacillus gasseri* in alginate-chitosan capsules and they observed that after 28 days of storage, the cellular viability decreased from 10^9 to 10^7 CFU ml^{-1}.

In conclusion, alginate/gelatin beads encapsulating probiotic *Lact. rhamnosus* at concentrations of 1 % w/v of alginate and 0.1 % w/v of gelatin resulted in formation of beads containing 10^9 CFU g^{-1}. The microparticles were spherical with slightly roughened surfaces, and interaction between polymers was confirmed by FTIR and thermal analysis. Beads maintained the cell survival of *Lact. rhamnosus*, reaching 10^5 CFU g^{-1} after 4 months. Thus, this formulation could be considered promising in the production of microcapsules containing probiotics because even after 4 months of storage a mass of 10 g of microcapsules would be enough to reach the minimum quantity recommended by FAO.

ACKNOWLEDGMENTS

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for the financial support.

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