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STUDY OF DIFFERENT ENCAPSULATING AGENTS FOR THE MICROENCAPSULATION OF VITAMIN B12

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Abstract

Recently, the studies about vitamin B12 increased due to the high number of people who can develop vitamin B12 deficiency, namely: vegetarians, pregnant women or with vitamin B12 malabsorption. One solution to correct the low nutritional intake of vitamin B12 can be using food supplements or pharmaceuticals, based on the vitamin B12 microencapsulation. In the present research, the vitamin B12 microencapsulation and the controlled release of fresh and 4 months' storage samples of vitamin B12 microcapsules were studied. The microcapsules were prepared using a spray-drying technique, and 7 biopolymers were used as encapsulating agents: arabic gum, sodium alginate, carrageenan, maltodextrin, modified starch, xanthan and pectin. The product yield of the spray-dryer ranged from 20 to 50%. The microparticles were also characterized in terms of size and morphology. The vitamin B12 release profiles from microcapsules were assessed by spectrophotometric analysis, at 361.4 nm, in deionized water at 22ºC and simulated gastric fluid at 37ºC. This study showed that the vitamin B12 microcapsules, with good stability properties, can be produced with several encapsulating agents and proved the possibility of releasing the vitamin in different periods of time.

Key words: A biopolymers, encapsulating agents, microencapsulation, spray drying, vitamin B12

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1. Introduction

Vitamins delivery systems used for health maintenance improved significantly in the last decades, due to the new food diet varieties and the challenge of treating diseases in a fast and less painful way possible. Daily administration of vitamins is mandatory (Ball, 2006; Pressman and Buff, 1997) therefore it is a constant "battle" for the industry fields to produce new food and pharmaceutical products with vitamin content (Dordevic et al., 2014; Teleki et al., 2013). The main problem of these essential organic compounds is their stability, so a protective system to keep their properties active and to avoid any type of degradation is necessary (Teleki et al., 2012).

Microencapsulation is a method widely used for drug controlled delivery and is suitable also for vitamins, since it was tested with success for many sensitive bioactives (Abbas et al., 2012; Carvalho et al., 2016; Dordevic et al., 2014; Estevinho et al., 2014a; Estevinho et al., 2016; Gonçalves et al., 2017; Rosiñski et al., 2008; Teleki et al., 2013).

This research work chose vitamin B12 as a model core material to be tested by spray-drying technique with several encapsulating agents within the biopolymers class. Known also as cyanocobalamin, vitamin B12 is a water-soluble vitamin, stable in normal conditions if not exposed to direct light (Ball, 2006; Combs, 2008; Eitenmiller and Landen, 1999; Pressman and Buff, 1997).

In the case of balanced diet, which includes also products of animal origin, people are rarely found to suffer from vitamin B12 deficiency, since the human body can store more than the required amount. However, vegetarians and pregnant women or during lactation are predisposed to suffer from this type of

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deficiency. For them and those patients who suffer from vitamin B12 malabsorption, a way to correct the level of vitamin must be found (Combs, 2008; Herrmann and Geisel, 2002; Longmore et al., 2014; Okuda, 1999; Robert and Brown, 2003; Watanabe et al., 2013).

Spray-drying is known as the most used microencapsulation method for food industry applications. Stable powders can be obtained from a one-step process of atomization. Also because of the low cost, continuous work mode, easy handling of equipment, spray-drying process is preferred among other microencapsulation techniques (Abbas et al., 2012; Comunian and Favaro-Trindade, 2016; Desai and Park, 2005; Gharsallaoui et al., 2007; Oliveira et al., , 2010; Teleki et al., 2013).

There is a big interest in changing the synthetic materials used for microencapsulation, so biopolymers will be a good alternative (Estevinho et al., 2012; Paiva et al., 2015). The option of using biopolymers, as encapsulating agents for the spray-drying process, can bring several advantages: bioavailability, biocompatibility, stability and no toxicity (Fathi et al., 2014; Freiberg and Zhu, 2004). For all these reasons, biopolymers can be considered suitable for the protection of vitamin B12, and further use of the microcapsules for applications of food and pharmaceutical industry, since the consumer is not exposed to any risks (Dordevic et al., 2014; Murugesan and Orsat, 2012).

The aim of this study was to investigate possible shell materials suitable for vitamin B12 oral delivery systems. These biopolymers materials are carbohydrates of different origin plants: arabic gum, maltodextrin and pectin; marine: sodium alginate and carrageenan; and also microbial origin: xanthan (Dordevic et al., 2014; Oliveira et al., 2010; Teleki et al., 2013). Microparticles were prepared by spray-drying method and then characterized regarding their size and surface aspect, using laser granulometry analysis and scanning electron microscopy (SEM). Finally, release profiles were evaluated by spectrophotometric analysis: tests were performed in deionized water at ambient room temperature (22ºC) and in simulated gastric fluid (SGF) at the human body temperature (37ºC).

The microencapsulation of vitamin B12 can be an option to integrate the vitamin in products that belong to the class of food supplements and pharmaceuticals, like: enriched food products, instant drinks, effervescent capsules, ready to eat cereal-bars, and multivitamin compressed caps.

2. Material and methods

2.1. Material

High purity and pharmaceutical grade reagents were selected for this work. Vitamin B12 was provided from Sigma-Aldrich, China (V2876, Lot # MKBQ9972V). Arabic gum from acacia tree was acquired from Fluka, Germany (30888, Lot #BCBK8649V), pectin from apples was obtained from Sigma-Aldrich, Switzerland (101582340 76282, Lot #BCBN5335V) and modified starch from Alfa Aesar GmbH&Co KG, Germany (36673, Lot D04X013). From Sigma-Aldrich, USA were purchased: sodium alginate (1001503523 180947, Lot # MKBH8463V), maltodextrin (1001841656 419672, Lot # MKBN6629V), carrageenan (1001761179 C1013 - 1006, Lot # SLBH9868V) and xanthan gum from *Xanthomas campestris* (1001900732 G1253, Lot # MKBQ9467V).

For the formulation of the simulated gastric fluid, hydrochloric acid from Sigma-Aldrich (25,814-8) and sodium chloride from AppliChem Panreac ITW Companies (131659.1211, Lot 0000542745) were used.

2.2. Preparation of solutions

A different spray-drying feed solution was prepared for each one of the 7 encapsulating agents. Solutions with a content of 1% (w/V) encapsulating agent (placed under stirring for 2 hours at a speed of 1200 rpm), and solutions with 2% (w/V) of vitamin B12 (mixed in the shaker for 10 minutes) were prepared.

For the microencapsulation experiments, the encapsulating agent samples were mixed with vitamin B12 samples to obtain feed solutions for the spray-dryer. 100 mL of encapsulating agent solution were mixed with 10 mL of vitamin B12 solution. The stirring was made for 30 minutes at a speed of 500 rpm. Feed solutions without vitamin content were prepared for the analysis of empty microcapsules.

The formulation of simulated gastric fluid (SGF) followed the European Pharmacopeia 7.0 (2010). A stock solution of SGF (1 L - aqueous solution) was prepared with 2.0 g of sodium chloride and 7.0 mL of hydrochloric acid 37%. Additional pH corrections were done with a pH-meter until a final pH value of 1.2 was reached.

All solutions were prepared at room temperature with deionized water.

2.3. Spray-drying process

The microparticles (empty and with vitamin B12) were prepared by a spray-drying technique in a Mini Spray Dryer B-290 from BÜCHI (Flavil Switzerland) with a standard 0.5 mm nozzle. For the shell materials, the following 7 biopolymers were used: arabic gum, sodium alginate, carrageenan, maltodextrin, modified starch, xanthan and pectin.

Each solution was fed up under the following experimental conditions: solution and air flow rates - 4 mL/min (15%), 32 m^3/h (80%), air pressure and inlet temperature - 6 bar and 120ºC, respectively. The outlet temperature varied between 56 and 67ºC, in the case of vitamin B12 microcapsules, and between 60 and 68ºC, for the empty microcapsules. The same experimental conditions were applied for all the samples and were selected based on the literature and

some previous studies (Abbas et al., 2012; Casanova et al., 2016; Estevinho et al., 2013b; Estevinho et al., 2016).

The final products were stored and kept for further tests at 4ºC, protected from light, to avoid degradation processes.

2.4. Microparticles characterization

The characterization of microparticles followed 2 types of analysis: surface morphology and particle size distribution. The surface morphology was examined by Scanning Electron Microscopy (SEM) and was performed with a Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M equipment (Eindhoven, The Netherlands) at Centro de Materiais da Universidade do Porto (CEMUP). The powder samples (microparticles) were previously fixed on a brass stub using double-sided adhesive tape and then were made electrically conductive by coating, in vacuum, with a thin layer of gold in a Jeol JFC 100 equipment.

For the determination of particle size distribution, a Coulter-LS 230 Particle Size Analyzer (Miami, USA) was used. By laser granulometry, particles were characterized by number and volume average. For each sample an average of three 30 seconds runs was set. The procedure was made after each sample was ultrasound-irradiated, and ethanol was used as dispersant solution to avoid the microparticles aggregation.

2.5. Analytical method for controlled release profile studies

An analytical method was chosen to confirm, by spectrophotometry, the presence of vitamin B12, as an active core inside the microcapsules. The release tests were carried out in 2 types of dissolution mediums at different temperatures: deionised water at 22ºC and SGF at 37ºC. The evaluation of vitamin B12 was done by measuring the absorbance with a spectrophotometer from Sarspec SPEC RES+ UV/VIS (Portugal), equipped with an external device for heating and stirring of the analyzed solution. For each test, readings were done in the UV domain at 361.4 nm wavelength and the data acquisition was set for continuous recording every 30 seconds in the case of the following encapsulating agents: arabic gum, sodium alginate, carrageenan, maltodextrin, xanthan and pectin, and every 5 seconds just for modified starch.

This method was first validated to prove the possibility of running the release studies. 13 standard solutions, in the range of 0.0025 g/L to 0.1000 g/L, were analyzed at room temperature. A linear calibration curve was obtained, with a good correlation coefficient R^2 = 0.9918. The limit of detection (LOD) was estimated to be 0.0563 µg/mL and the limit of quantification (LOQ) 0.1876 µg/mL.

For the release studies, 3 mg of microparticles were placed inside the cuvette filled with 3 ml of analyzing liquid. The quantity of vitamin B12 microcapsules was selected according to the methodology described in a previous work (Estevinho et al., 2016), being determined by mass balance of reagents used, assuming that during the spray-drying process the ratio of core material/encapsulating agent remained constant. Continuous stirring mode was set on for the cuvette and, depending on the type of analysis, temperature was adjusted (first round of experiments at 22ºC, second one at 37ºC). Tests were considered finished when all the vitamin content was released, corresponding to the stabilization in time of the value of absorbance. Samples were stored protected from light, in the fridge (4ºC) for 4 months and then were tested as described in this section, to check the stability of the samples.

All release tests and the calibration solutions were performed in triplicate, showing coefficients of variation smaller than 10%.

3. Results and discussion

3.1. Product yield

The product yield (%) of the spray drying process was determined for each system studied and was expressed as a ratio of recovered powder reported to the introduced amount of raw materials (Fig. 1).

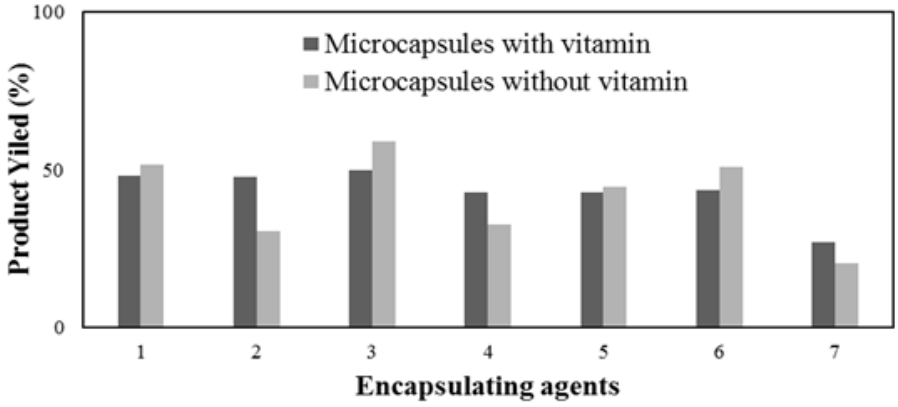

Fig. 1. Product yield for spray-drying process
(1 – arabic gum, 2 – carrageenan, 3 – maltodextrin, 4 – modified starch, 5 – pectin, 6 – sodium alginate, 7 – xanthan)

The results obtained for both types of microparticles are between 27 and 50% for vitamin B12 microparticles, and between 20 and 59 % for empty ones, as shown in Fig. 1. These values are acceptable compared with previous works: 41 – 56% for vitamins B12 and C (Estevinho et al., 2016), and 30 – 50% for food flavours (Estevinho et al., 2013a) and enzymes (Estevinho et al., 2014a; Estevinho et al., 2015). The only encapsulating agent having a low product yield is xanthan: 27% for microparticles with vitamin B12 and 20% for the empty ones.

3.2. Microparticles characterization in terms of size and morphology

All microparticles, with or without vitamin B12, were characterized regarding their surface morphology and their size. The specific morphology for each biopolymer was observed in SEM images. In Fig. 2, microcapsules with spherical shape and smooth surface, corresponding to sodium alginate, carrageenan, maltodextrin and pectin, are presented. Microcapsules for the other 3 encapsulating agents, arabic gum, modified starch and also xanthan, presenting spherical shape with rough surface, can be observed in Fig. 3. The morphology found in the first group of microparticles was also obtained for vitamin B12 microparticles with modified chitosan and sodium alginate (Estevinho et al., 2016) and with poly (acrylic acid)-cysteine (Sarti et al., 2012).

On the other hand, similar morphology to the second group of encapsulating agents was obtained with chitosan capsules (Estevinho et al., 2016). Size characterization of microparticles performed by laser granulometry shows different sizes for microcapsules with and without vitamin B12 (Table 1).

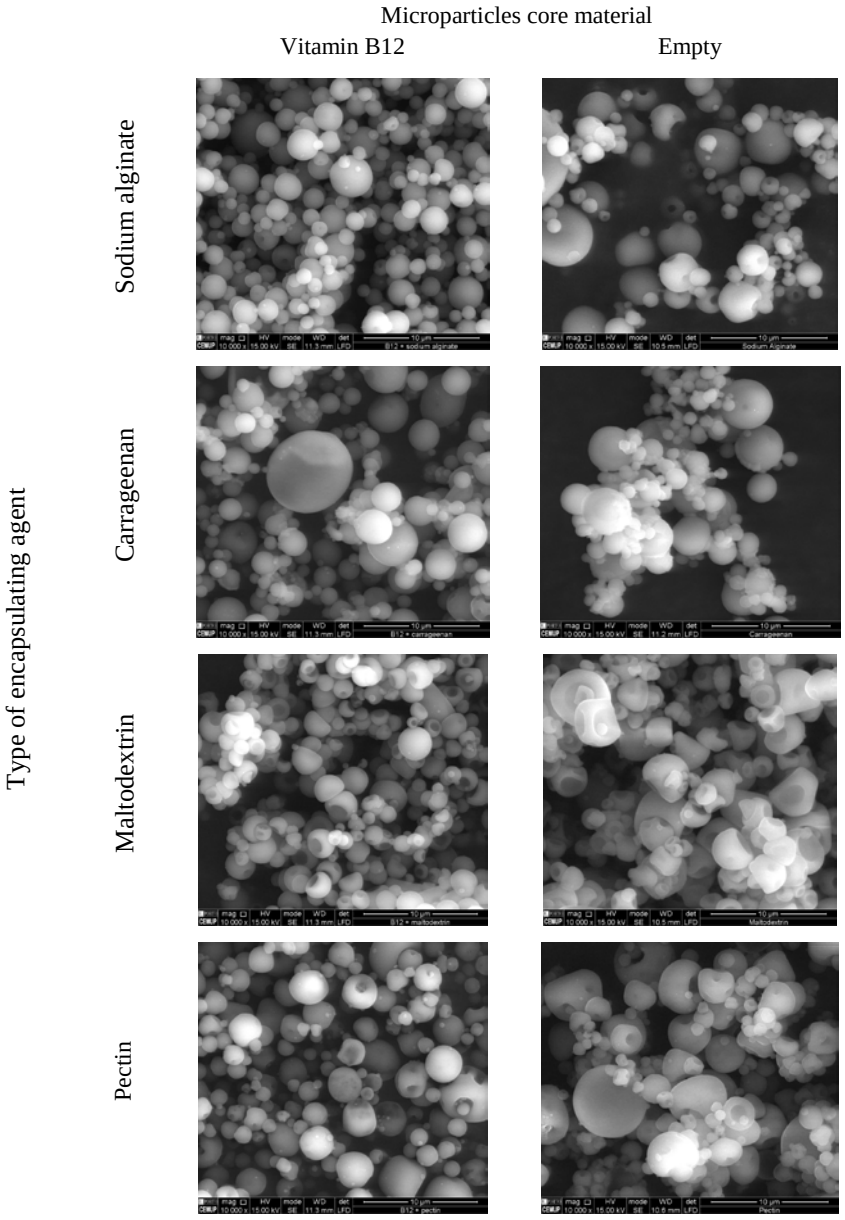

Fig. 2. SEM images of sodium alginate, carrageenan, maltodextrin and pectin microcapsules, with and without vitamin B12. Magnification = 10. 000 times, beam intensity (HV) 15.00 kV, distance between the sample and the lens (WD) around 10 mm

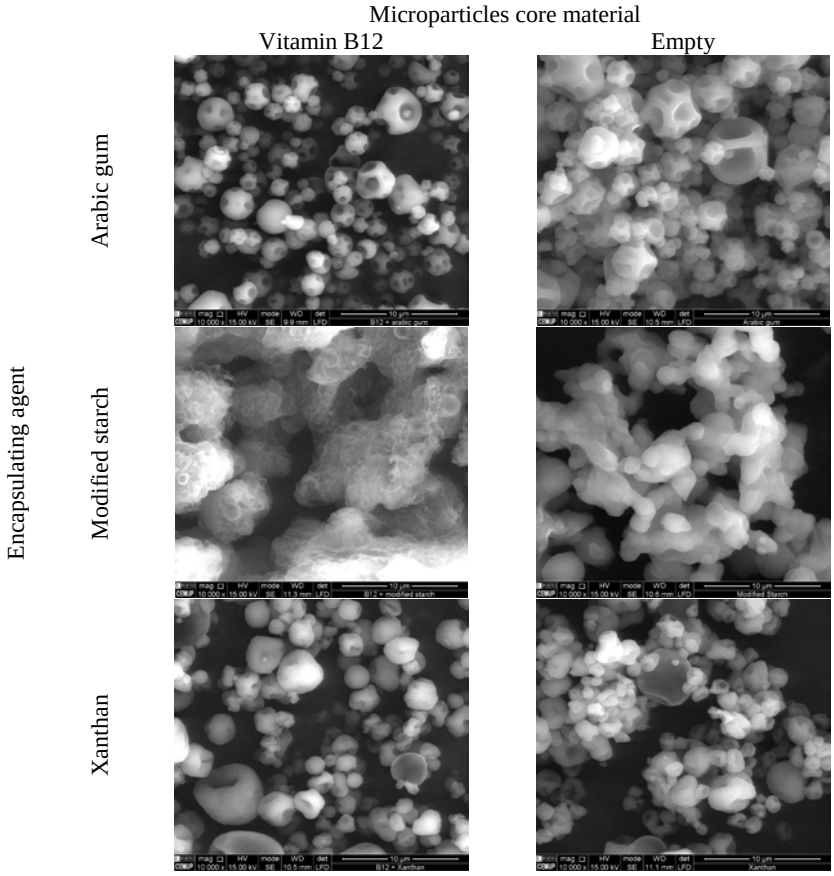

Fig. 3. SEM images of arabic gum, modified starch and xanthan microcapsules with and without vitamin B12. Magnification = 10. 000 times, beam intensity (HV) 15.00 kV, distance between the sample and the lens (WD) around 10 mm

Table 1. Particle mean size by laser granulometry considering volume and number distribution for microcapsules with vitamin B12 and for empty microcapsules

		Differential volume distribution (µm)		***Differential number distribution (µm)***	
		Microcapsule core material			
		Vitamin B12	***Empty***	***Vitamin B12***	***Empty***
Encapsulating agent	Arabic gum	3.17	4.22	0.96	0.57
	Sodium alginate	3.35	10.18	0.93	0.60
	Maltodextrin	4.83	-	0.94	-
	Carrageenan	5.16	7.44	0.98	0.54
	Xanthan	6.58	7.56	0.93	0.72
	Pectin	6.67	4.66	0.99	1.77
	Modified starch	32.17	42.00	2.74	3.62

For microparticles with active core material the average diameter according to volume distribution increases from 3.17 up to 6.67 µm in this order: arabic gum, sodium alginate, maltodextrin, carrageenan, xanthan and pectin. As for the number distribution, the value of average diameter is maintained constant at approximately 1 µm, suggesting aggregation processes, fact highlighted also by the SEM images.

Empty microcapsules present mean sizes, for volume distribution, higher than the microcapsules with vitamin content. Regarding the number distribution of empty microcapsules, it can be observed that the behavior is the opposite; all the values are smaller than 1 µm excepting the sizes of pectin and modified starch capsules, 1.77 and 3.62, respectively. It was not possible to obtain the size distribution of the empty maltodextrin capsules.

Regarding modified starch microparticles, the sizes found are much higher than for the other encapsulating agents, even in the case of empty microcapsules. As shown in the Table 1, for vitamin B12 capsules the obtained values are 32.17 µm for volume distribution and 3.62 µm for number distribution. SEM images for this biopolymer, Fig. 3, show a significant particle aggregation.

3.3. Controlled release studies for fresh and 4 months samples

The controlled release profiles of vitamin B12 from biopolymers microcapsules were evaluated based on spectrophotometric measurements done at vitamin's specific wavelength: 361.4 nm. Release tests were performed using two different solvents:

deionized water at room temperature to simulate the main conditions at food industry, and SGF solution at 37ºC to recreate human gastric conditions. Different release profiles behaviors were observed, demonstrating the effect of the type of microparticle, pH and temperature. The evaluation of the release of vitamin B12 was considered to be complete when the value of the absorbance remained stable.

Taking into account the different types of found morphologies, the release profiles are presented in two figures. In Fig. 4, one can observe the release profiles for the following encapsulating agents: sodium alginate, carrageenan, maltodextrin and pectin. In this figure, the profiles obtained in the two solvents, water and SGF, for fresh samples, are presented. In Fig. 5 the same is made for the other three encapsulating agents: arabic gum, modified starch and xanthan.

As can be observed in Figs. 4 and 5, microparticles tend to dissolve gradually, some of them slowly, some moderately or very fast. The microparticles of arabic gum, carrageenan, maltodextrin and modified starch present fast releases, very fast in the case of modified starch, and similar profiles. In all these cases, the release profiles in water and in SGF are almost equal and the release is completed in less of 5 min. The type of release is provoked by the type of encapsulating agent used, the sensibility of the biopolymer to the change of the pH, and the type of interactions between the encapsulating agent and vitamin that are established. In these specific cases the release profile had the same behaviour in water and in SGF for each encapsulating agent used. So, for these specific cases the type of solvent is irrelevant. Regarding sodium alginate microparticles the release is moderate, less than 15 min in both solvents, and one observes a different profile in the release in water, this release being also slower.

In the case of pectin microparticles, the release is completed in less than 70 min in water, faster in SGF, but the release profile is similar for the two solvents. Xanthan showed to be the most sensitive polymer to pH and temperature changes. The release is slower, and the effect of the solvent is pronounced: the release changes from 1 h in SCF to about 15 h in water, and the profile is different.

Comparing Figs. 4 and 5, with Figs. 2 and 3, it is not possible to relate the morphology with the release profile. The properties of encapsulating agents will reflect on the capacity of entrapping the core material protecting it and avoiding mass losses over time. The encapsulating agent will also, affect, the morphology and size of the particles.

Another information that can be obtained from the release profiles is the encapsulation efficiency that is the percentage of drug that is successfully entrapped into the particle. About the encapsulation efficiency there are different methods and strategies to determine it: in this case and considering the specifications of our experimental design, the encapsulation efficiency can be calculated considering the release profiles, and corresponds to the amount of compound that is encapsulated in the time zero. For these experiments these values correspond to encapsulation ratios around 100% for the microparticles prepared with all the encapsulating agents, except for the case of the microparticles prepared with maltodextrin and modified starch and released in SGF, where this value is lower, around 60%.

Targeted delivery systems for bioactive compounds, like vitamin B12, can be a good solution for creating new food and pharmaceutical products. One of the big advantages of the microencapsulation is the protection. For example: a commercial instantaneous gelatin in powder can be fortified with vitamins; in this case a fast release is desired for the gelatin be homogeneous for the consumption. But during the time that it was in the package, stored, was protected from oxidation, light and interaction with other components. In other cases, as for example in pharmaceutical products, a slow release can be desired, in order to promote a more efficient absorption in the intestinal tract.

Therefore, it is important to choose effective wall materials capable to prevent or minimize the losses until they are utilized. This research work showed good results in what concerns the capsule stability for samples stored for 4 months. In Table 2, the mass loss of vitamin B12 is presented in percentage for each wall material. The stability of the microparticles over the time was evaluated with SGF. The losses of vitamin ranged from 6.2 to 22.4%, the smallest value corresponding to arabic gum and the highest to pectin.

4. Conclusions

The encapsulation of vitamin B12 was performed using 7 different biopolymers as encapsulating agents, using a spray-drying technique. The product yield for the spray-drying process is between 27 and 50% depending on the encapsulating agent.

Good microparticles were produced, with different morphologies and sizes. All particles show a spherical shape, the particle surface being smooth for sodium alginate, carrageen, maltodextrin and pectin microparticles, and rough for the other 3 biopolymers, arabic gum, modified starch and xanthan. The average diameter of vitamin B12 microparticles according to volume distribution ranges from 3.17 to 6.67 μm.

Different release profiles were found, depending on the encapsulating agent and the release conditions, pH and temperature.

In terms of stability over time it can be concluded that the shelf life of microcapsules can be extended at least until 4 months. The encapsulating agents used show good protective effect. This study proved that vitamin B12 can be microencapsulated by spray-drying technique using several biopolymers as encapsulating agents.

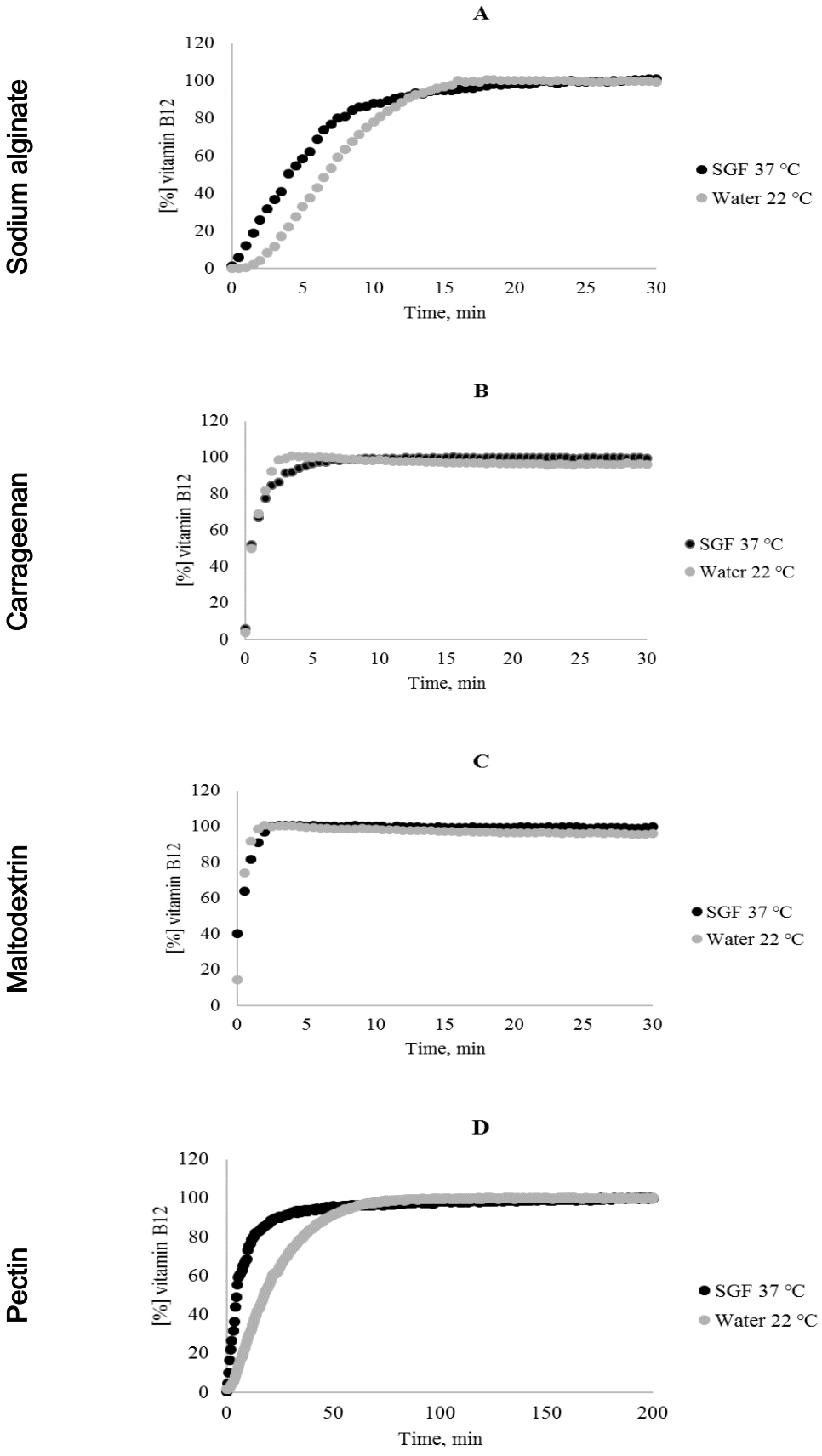

Fig. 4. Vitamin B12 release profile performed in: SGF at 37ºC and deionized water at 22ºC from microcapsules of (A) – sodium alginate, (B) – carrageenan, (C) – maltodextrin and (D) – pectin

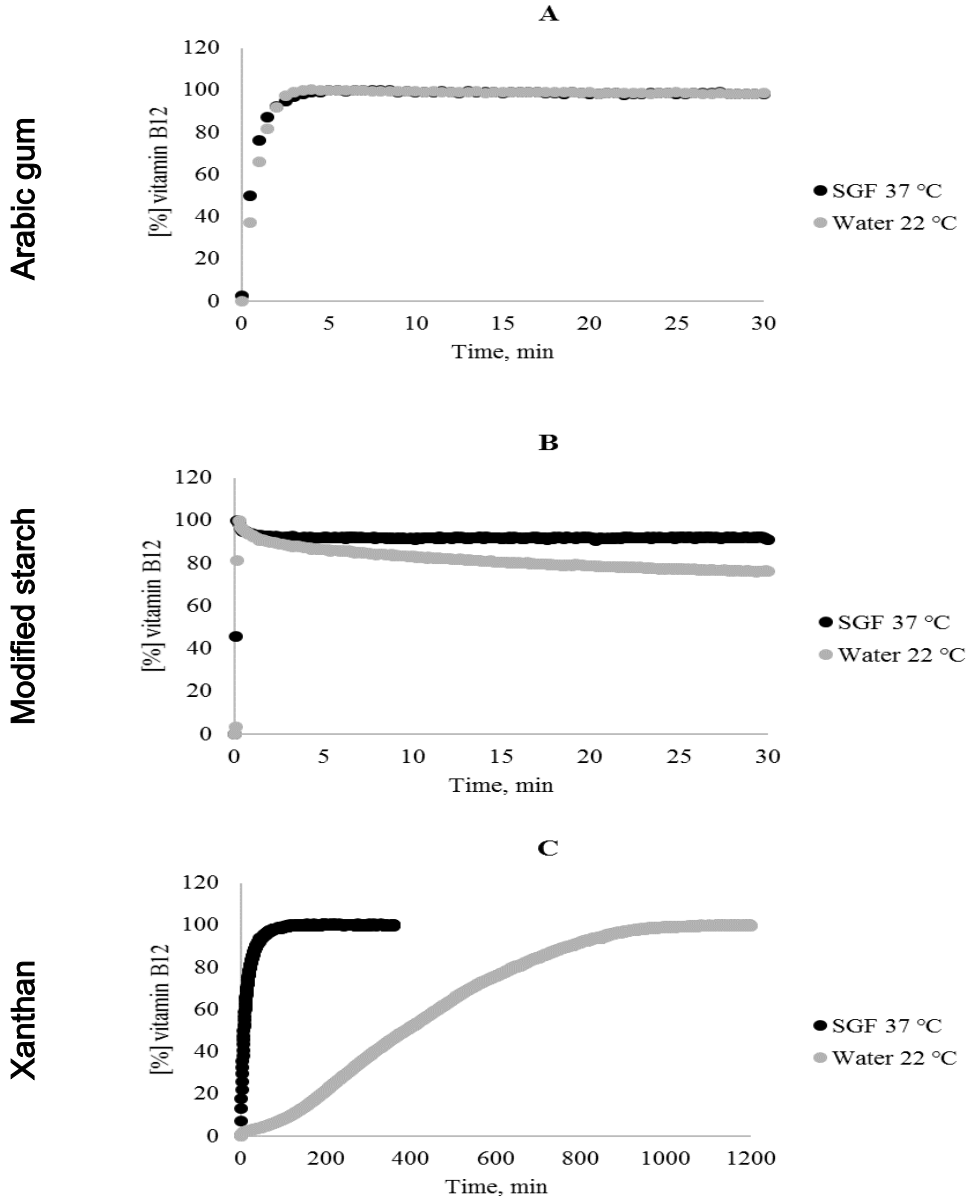

Fig. 5. Vitamin B12 release profile performed in: SGF at 37ºC and deionized water at 22ºC from microcapsules of (A) – arabic gum, (B) – modified starch and (C) – xanthan

Table 2. Mass loss of vitamin B12 after 4 months of storage in percentage for each type of biopolymers used as an encapsulating agent

Encapsulating agent	***Mass loss of vitamin B12 (%) after 4 months of storage for samples released in SGF:***
Arabic gum	6.2
Sodium alginate	7.7
Carrageenan	12.0
Maltodextrin	11.7
Modified starch	7.6
Pectin	22.4
Xanthan	12.7

The microparticles prepared with different encapsulating agents present different behaviours, and this fact can lead to different future applications, according to what it is expected from the final product (size and morphology of the microcapsules, needed time for the complete release of vitamin from the protective layer, type of solvent used to evaluate the release). Further, it is shown that these products are a promising solution to be used for the formulation of oral delivery systems with good stability properties.

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