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ORIGINAL PAPER

Microencapsulation of a Natural Antioxidant from Coffee—Chlorogenic Acid (3-Caffeoylquinic Acid)

B. Gonçalves[1] · M. Moeenfard[1] · F. Rocha[1] · A. Alves[1] · B. N. Estevinho[1] · L. Santos[1]

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Abstract Chlorogenic acids, the main polyphenolic group present in coffee, which include the caffeoylquinic acids, are recognized as antioxidants with growing interest in pharmacological, cosmetic, and food applications. However, they can be easily oxidized and they are also very unstable when exposed to high temperatures. Therefore, they can suffer transesterification reactions during storage or food processing, limiting their applications. Nevertheless, this situation can be overcome or minimized by microencapsulation. The purpose of the present study was to prepare by a spray-drying process sodium alginate and modified chitosan microparticles with chlorogenic acid (3-CQA), characterize them (morphological analysis), and evaluate the release profile of 3-CQA from the microparticles in in vitro studies. Furthermore, their antioxidant activity and moisture content were determined. The results address the success of chlorogenic acid microencapsulation, resulting in stable microparticles with controlled release properties and good antioxidant activity, suggesting increasing applications in food and pharmaceutical industry.

Keywords Antioxidant activity · Biopolymers · Chlorogenic acid · Controlled release studies · Microencapsulation · Spray drying

✉ B. N. Estevinho
berta@fe.up.pt

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

Introduction

Coffee is one of the most consumed and commercialized food products in the world. From a chemical point of view, it contains several biologically active compounds (Moeenfard et al. 2014), as polyphenols, with proven healthy effects in the human body (Niseteo et al. 2012). Chlorogenic acids (CGAs) are the main group of polyphenols present in coffee (Sato et al. 2011). CGAs are phenolic compounds naturally formed by the esterification of cinnamic acids, such as caffeic, ferulic, and *p*-coumaric acids, with (−)-quinic acid (QA) (Ayelign and Sabally 2013; Duarte et al. 2010; Monteiro and Farah 2012). CGAs are usually classified in three groups namely, caffeoylquinic acids (CQAs), dicaffeoylquinic acids (diCQAs), and feruloylquinic acids (FQAs), which are also known due to their contribution to the astringency and bitterness of the final beverage (Ayelign and Sabally 2013; Duarte et al. 2010; Monteiro and Farah 2012).

Apples, pears, berries, and prunes are some other examples of their natural occurrence, although green coffee beans are considered the richest natural source of CGAs with a content of 5–12 g/100 g (Farah et al., 2008).

These compounds are highlighted due to their health benefits on the human body (Cano-Marquina et al. 2013). CGAs present relevant nutritional and functional properties mainly associated with their antioxidant activity (Farah and Donangelo 2006; Monteiro and Farah 2012). Moreover, CGAs present other important activities such as anti-inflammatory, antimicrobial, and antiviral. Also, their contribution in the prevention of several diseases associated with oxidative stress, such as cancer, premature aging, strokes, Alzheimer's, Parkinson's, and other cardiovascular and neurodegenerative diseases have been also documented in literature (Belay and Gholap 2009; Farah et al., 2008; Perrone et al. 2008).

Considering all these beneficial properties, CGAs has gained a growing interest for pharmacological, cosmetic, and

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food applications. However, due to their structural and chemical nature, CGAs can easily undergo oxidation. Furthermore, they are unstable under high temperature conditions and they may suffer from transesterification reaction during storage or food processing, limiting their food and pharmaceutical applications (Farah and Duarte 2015; Komes and Bušić 2014; Shi et al. 2007). In addition, thermal processing of food products rich in CGAs may originate the formation of acrylamide (Nallamuthu et al. 2014).

So, the supplementation of food and pharmaceutical products with CGAs requires a formulation able to maintain and protect their structural integrity until their consumption, mask their taste, increase their bioavailability, and finally control their release. The solution for this can be obtained by microencapsulation.

Microencapsulation has been widely reported as an efficient solution to solve problems associated to the manipulation and incorporation of CGAs in different formulations/products and has emerged as a promising delivery system to stabilize and protect CGAs in order to overcome some limitations associated to their industrial application and commercialization. Microencapsulation is a well-established technique which protects the core material against moisture, light, and oxygen through formation of a physical barrier between the core materials and the surrounding environment (Fang and Bhandari 2010; Nesterenko et al. 2013). On the other hand, microencapsulation can improve compounds with new physical characteristics, mask their unpleasant taste, and also allow a controlled release of core material (Fang and Bhandari 2010). During the last years, several encapsulated compounds (antioxidants, vitamins, enzymes, minerals, lipids, probiotics, and others) have been used in food industry to overcome the drawback of application of the free compounds (Carvalho et al. 2015; Estevinho et al. 2014a; Estevinho et al. 2013a; Fang and Bhandari 2010; Gonçalves et al. 2016).

Microparticles can be produced by several techniques (Fang and Bhandari 2010). Spray drying is the most used encapsulation technique in food industry due to its low cost, availability of equipment, and efficiency (Estevinho and Rocha 2016; Estevinho et al. 2013b; Munin and Edwards-Lévy 2011). Nevertheless, one of the biggest challenges in microencapsulation of compounds is the utilization of food-grade encapsulating materials, in order to be compatible with the demands and standards of food industry. The application of several biopolymers as wall-forming materials in microencapsulation of food ingredients has been reported in literature (Estevinho et al. 2013b). Among them, sodium alginate and chitosan carry several benefits such as high biocompatibility, biodegradability, non-toxicity, among other biological advantages (e.g., anticholesterolemic properties of the chitosan).

There are some studies about the encapsulation of CGAs (Lorentz et al. 2012; Nallamuthu et al. 2014; Qi et al. 2010; Ramírez-Ambrosi et al. 2014; Shi et al. 2007); however, the applicability of sodium alginate and modified chitosan (water-soluble chitosan, prepared by carboxylation) as non-toxic, biocompatible, and biodegradable encapsulating agents for the microencapsulation of chlorogenic acids using a spray-drying process was not reported up to date. Therefore, the use of a spray-drying technique associated to natural biopolymers can overcome the limitations of the use of free chlorogenic acids in healthy products, improving their consumption with their incorporation in microencapsulated formulations (Estevinho et al. 2015; Estevinho et al. 2016).

Thus, the present study aimed the microencapsulation of a chlorogenic acid (3-CQA) into sodium alginate and modified chitosan-loaded microparticles by spray drying, with subsequent characterization of the microparticles and also evaluation of its release profile and antioxidant activity.

Material and Methods

Chemicals

All reagents were of analytical grade purity. Chlorogenic acid standard (3-caffeoylquinic acid, C16H18O9, CAS 327-97-9) (≥98% purity) was acquired from Sigma-Aldrich Chemical Co. (MO, USA). Sodium alginate (alginic acid, sodium salt) obtained from Sigma-Aldrich Chemical Co. (USA) and modified chitosan (pharmaceutical grade water-soluble chitosan) from China Eastar Group (Dong Chen) Co., Ltd. were used as encapsulating agents. Modified chitosan (water soluble) was produced by carboxylation and had a deacetylation degree of 96.5% and a viscosity of 5 mPa s^{-1} (1%, 25 °C). Ethanol (96%) was obtained from Aga (Prior Velho, Portugal). HCl (≥37% purity), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulfate, and trolox were obtained from Sigma-Aldrich (St. Louis, MO, USA). Deionized water was obtained at the laboratory using MilliporeTM water purification equipment through a 0.45 μm filter (Massachusetts, USA).

Equipment

A 420-W ultrasonic bath (J.P. Selecta, Barcelona, Spain) was used for preparation of 3-CQA stock solution.

Microencapsulation was performed using a mini spray-dryer BÜCHI B-290 (Flawil, Switzerland) with a standard 0.5 mm nozzle.

The size distribution of the microparticles was evaluated by laser granulometry using a Coulter LS 230 Particle Size Analyzer (Miami, FL, USA).

The morphological analysis of the obtained microparticles was performed by SEM using a Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M. Samples were coated by gold pulverization under vacuum in a Jeol JFC 100 apparatus.

The concentration of chlorogenic acid in the solution was measured based on absorbance values, read using a UV-Vis spectrophotometer (Jasco V-530).

Preparation of Microparticles by Spray Drying

The spray drying is a unit operation by which a solution/suspension/emulsion is atomized in a hot gas (generally air) to instantaneously obtain a powder and by this way to form the microparticles. The process of spray drying commonly implies several steps (Estevinho et al., 2013a; Estevinho et al. 2013b; Gonçalves et al. 2016, 2017). The first step is dissolving, emulsifying, or dispersing the active compounds (core material) to prepare the "feed solution" that can be a solution, an emulsion or a suspension (Đorđević et al. 2014).

In this work, the feed solution was a solution constituted by the core compound (3-caffeoylquinic acid) and the biopolymer (sodium alginate or modified chitosan). A stock solution containing 3-CQA was prepared with deionized water at a concentration of 10 g L^{-1} and homogenized in an ultrasound bath for 5 min. Each encapsulating agent solution (modified chitosan or sodium alginate, 10 g L^{-1}) was prepared with deionized water and completely dissolved under agitation at 500 rpm at room temperature (21 °C). Under magnetic stirring at room temperature, 2 mL of the chlorogenic acid stock solution (10 g L^{-1}) was added to 100 mL of each encapsulating agent solution and kept under agitation for 30 min before being fed to the spray dryer. The same procedure was performed to obtain the modified chitosan and sodium alginate microparticles.

After, the following main steps are involved: atomization, formation of the droplet/air contact, evaporation of the water, and dry product/humid air separation (Gharsallaoui et al. 2007). The same operating conditions were used in the spray dryer for both types of the prepared microparticles. The operational conditions of the spray dryer used in these steps are presented in Table 1. The outlet temperature (T_{out}) is a consequence of the other experimental conditions and also of the solution properties and was around 67 °C. Microparticles were collected and stored in falcon tubes at 4 °C for further analysis.

Table 1 Experimental conditions of spray-drying process

Spray-dryer parameters	Values
Tin[a] (°C)	115
Tout[b] (°C)	67
Solution flow rate (%)	15
Aspiration (%)	100
Nozzle (mm)	0.5
Pressure (bar)	6

[a] Inlet temperature

[b] Outlet temperature

Characterization of the Microparticles

Product Yield (%)

The product yield (%) was calculated for each microencapsulation experiment and was expressed as the ratio of the mass of powder (microparticles) obtained in the spray-dryer output and the solid content of the initial feed solution using the following definition:

$$\text{Yield}\ (\%) = \frac{\text{Mass of powder obtained at the spray dryer}}{\text{Solid content of the initial feed solution}} \times 100 \quad (1)$$

Particle Size Distribution

The size distribution of the microparticles produced with sodium alginate and modified chitosan was evaluated by laser granulometry. Each small powder sample was dispersed in ethanol and ultrasound-irradiated over 10 s to avoid particle agglomeration. Characterization was done by number and volume average and the results were obtained as an average of two 60 s runs.

Scanning Electron Microscopy Analysis

The morphological analysis of the obtained microparticles was performed by SEM. Samples (microparticles) were coated with gold by pulverization under vacuum and analyzed by SEM for surface structure observation. All the SEM analyses were performed at Centro de Materiais da Universidade de Porto (CEMUP).

Controlled Release Studies (In Vitro)

Analysis of 3-CQA

Analysis of 3-CQA was performed by UV-Vis spectrophotometry at 324 nm. Calibration curve was built using seven concentration levels of 3-CQA in the range of 0.5–15 mg L^{-1}. The effect of the pH was evaluated in the preparation and validation of the calibration curve. No significant effect was observed for standards prepared with different pH values (2 and 5.6) for the same concentration of 3-CQA. The validation of the analytical method was done through the determination of the linearity, limit of detection (LOD), and limit of quantification (LOQ) and precision (inter- and intra-day).

The interference of the biopolymer solutions was tested at 324 nm, without presenting a significant signal.

Determination of the Release Profiles

The release profile of chlorogenic acid from sodium alginate and modified chitosan microparticles was obtained in aqueous solutions at two different pH values (2.0 and 5.6). Acidified

water at pH 2.0 was obtained by adding appropriate amount of HCl to distilled water under continuous stirring.

A preliminary mass balance was performed in order to estimate the adequate amount of microparticles to be used in the release studies; for this, the amount of chlorogenic acid contained in the produced microparticles was considered. To estimate this value, it was necessary to take into account the amount of reagents used, the ratio 3-CQA/encapsulating agent and the specifications of the spray-drying process. So, 3 mg of microcapsules were extracted in 4.5 mL of distilled water under two different pH values (2.0 and 5.6) during 24 h of agitation at 60 rpm. Samples were obtained in duplicate and were analyzed after time intervals of 0, 1, 2, 5, 10, 20, 30, and 45 min and 1, 2, 4, 6, and 24 h. So, the content of chlorogenic acid in the solution was determined at room temperature (21 °C), by UV-Vis spectrophotometry at 324 nm using the calibration curve previously validated.

Antioxidant Activity Assay

The antioxidant activity of the microparticles, free 3-CQA, and both biopolymers was determined by the ABTS radical scavenging assay as described in literature (Thaipong et al. 2006). Two stock solutions were prepared: 7.4 mM ABTS aqueous solution and 2.6 mM potassium persulfate aqueous solution. Then, the ABTS radical cation ($ABTS^{\bullet+}$) solution was obtained by mixing equal quantities (1 mL) of these two stock solutions. The mixture was allowed to stand in dark at room temperature during 12 h before use. This last solution, the $ABTS^{\bullet+}$ solution, was diluted by mixing 1 mL with 60 mL of ethanol in order to obtain an absorbance of 1.10 ± 0.02 AU at 734 nm (Thaipong et al. 2006). The samples (150 μL) were then mixed with the prepared solution (2850 μL) and allowed to react for 2 h in the dark. These samples consisted of 3-CQA-loaded sodium alginate and modified chitosan microparticles after 24 h release in aqueous solution (deionized water), and 3-CQA and sodium alginate and modified chitosan reference standards in solution in a concentration of 10.0 mg L^{-1}, prepared with deionized water. Analysis was performed by UV-Vis spectrophotometry at 734 nm and room temperature. Trolox was used as a reference compound, and a calibration curve was prepared, using five different concentrations (50–500 μM). The results were expressed in micromolar Trolox equivalents (TE). All measurements were performed in duplicate.

Moisture Content Assay

The moisture content of the produced microparticles was determined using the following expression:

$$\text{Moisture content}\ (\%) = \frac{\text{Mass of original sample} - \text{Mass of dried sample}}{\text{Mass of original sample}} \times 100 \quad (2)$$

Through this method, each sample of 3-CQA microparticles (sodium alginate and modified chitosan microparticles) was weighted (50 mg) in a crucible of porcelain and dried in oven at 105 °C until reaching a constant weight (duplicated assays) (Bradley, 2010). The results were presented as percentage of water content present in the microparticles.

Results and Discussion

The microparticles were prepared by a spray-drying technique that is flexible, offers substantial variation in matrix microencapsulation, and is adaptable to commonly used processing equipment and produces particles of good quality. The microparticles produced are normally matrix type (with the substance to encapsulate distributed in the shell) and the mechanisms of release involved are typically controlled by solvents action and by diffusion (de Azeredo, 2005). The first step of the microencapsulation process was dissolving the active compounds (core material) and the encapsulating agent to prepare the "feed solution" (Đorđević et al. 2014). After, the main following steps are involved: atomization, formation of the droplet/air contact, evaporation of the water and dry product/humid air separation (Estevinho et al. 2013b; Gharsallaoui et al. 2007; Vos et al. 2010). The microparticles formed were characterized and the main results are presented in the next subsections.

Characterization of the Microparticles

Product Yield (%)

A product yield of 41.1 and 39.3% was obtained for the production of sodium alginate and modified chitosan microparticles, respectively.

According to the type of the equipment and the low volumes of feed solution used, these values of product yield were expected because the small size of the obtained microparticles makes difficult the separation of the microparticles from the air in the cyclone (low cyclone efficiency) causing a decrease of the product yield. In fact, several authors have already reported a product yield between 30 and 70% (Casanova et al. 2016; Estevinho et al. 2014a; Estevinho et al. 2016; Estevinho et al. 2013a). Estevinho et al. (2014a) reported product yields of 36 and 59% for the microencapsulation of β-galactosidase with sodium alginate and modified chitosan, respectively, by spray drying.

Particle Size Distribution

Results obtained for particle size distribution in number (%) and in volume (%) of 3-CQA microparticles produced with

sodium alginate and modified chitosan are presented in Fig. 1a, b, respectively. According to Fig. 1, the average value of the particle size in volume distribution was around 3 μm for both types of samples (2.8 and 3.2 μm for sodium alginate and modified chitosan microparticles, respectively). On the other hand, considering the particle size distribution in number, the average particle size was less than 1 μm for the prepared microparticles, 0.7 and 0.9 μm for sodium alginate and modified chitosan, respectively. The size distribution profiles suggest the existence of a slight agglomeration of the particles. Additionally, the results proved that particle size, in the different distributions, was similar for the different types of the wall material used, since a similar mean diameter was obtained, thus suggesting that all the microparticles were formed by the same mechanism of microencapsulation.

Scanning Electron Microscopy

The chlorogenic acid-loaded microparticles were analyzed by SEM to determine their surface characteristics. SEM images of the microparticles prepared with sodium alginate and with modified chitosan are presented in Fig. 2.

It is possible to observe that spherical microparticles with smooth surface and regular shape were produced for both types of microparticles. However, modified chitosan microparticles showed to be more regular and with smoother surface than microparticles performed with sodium alginate. The surface of the microparticles depends on the encapsulating agent and the microencapsulation process used. The regular or irregular nature of the surface gives a first idea about the stability of the microparticles formed. On the other hand, smooth surfaces promote less interaction with other particles, which may be useful in certain applications.

Also, other authors already reported the same morphology for spray dried microparticles using sodium alginate and modified chitosan as wall materials (Estevinho et al. 2014a, 2014b; Estevinho et al. 2016).

Controlled Release Studies

Validation of the Analytical Methodology for 3-CQA Analysis

The concentration of 3-CQA was quantified by a linear calibration curve in the range of 0.5–15 mg L^{-1}, with R^2 equal to 0.999. The precision of the method was measured through inter- and intra-day precision at three concentration levels (2, 8, and 10 mg L^{-1}) in duplicate and the results are less than 5% in all analysis. LOD and LOQ of 0.09 and 0.31 mg L^{-1} were obtained, respectively.

Determination of the Release Profiles

The evaluation of the released 3-CQA was determined by UV-Vis spectrophotometry. Figure 3 presents the 3-CQA release

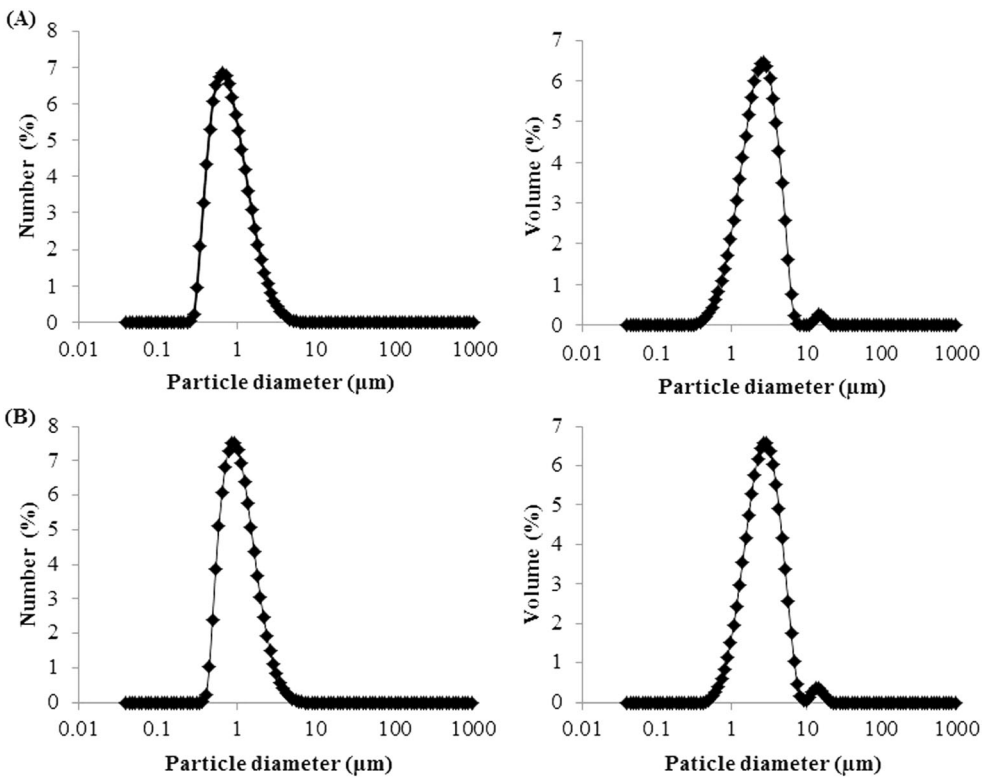

Fig. 1 Particle size distribution in number (%) and in volume (%) of chlorogenic acid (3-CQA) microparticles produced with **a** sodium alginate and **b** modified chitosan. The assays were done in duplicate ($n = 2$)

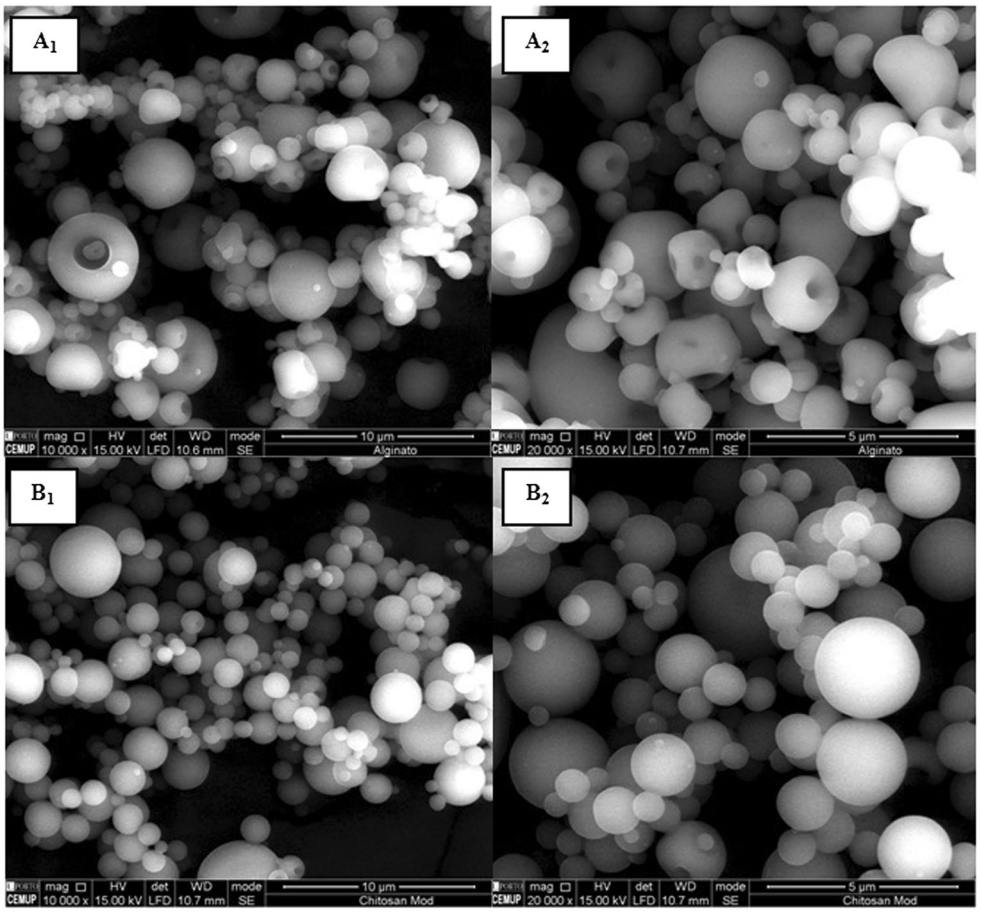

Fig. 2 SEM images of chlorogenic acid (3-CQA) microparticles prepared with **a** sodium alginate and **b** modified chitosan. Magnification of ×10,000 (A_1, B_1) and ×20,000 (A_2, B_2) beam intensity (HV) of 1500 kV, distance between the sample and the lens (WD) around 10 mm

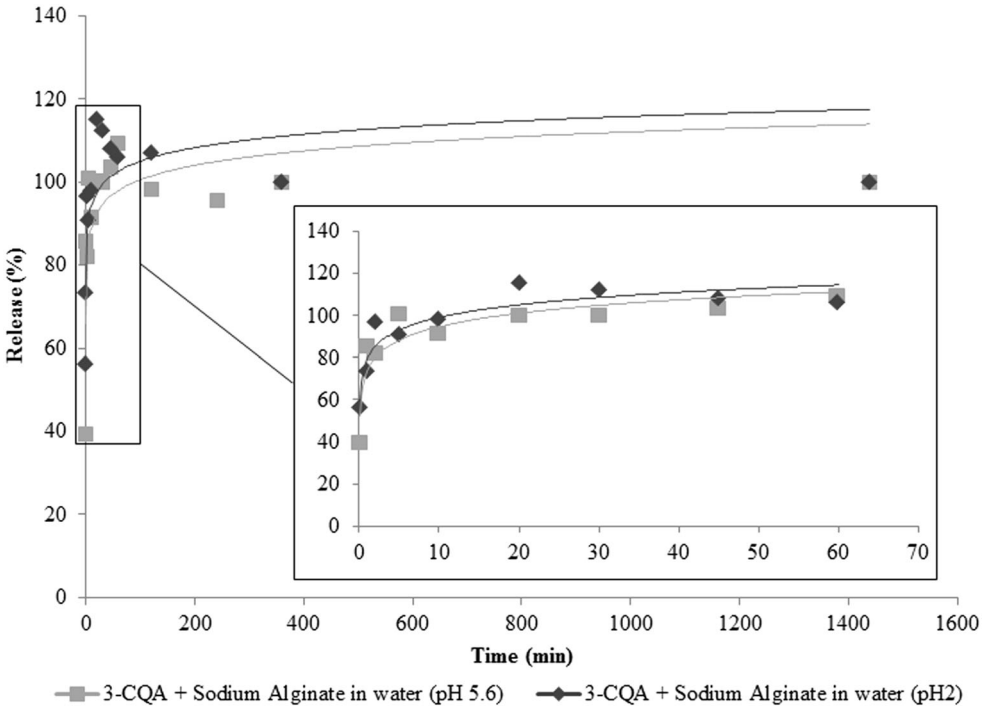

Fig. 3 Chlorogenic acid (3-CQA) release profile from sodium alginate microparticles in distilled water at two different pH values (pH 5.6 and pH 2.0). The assays were done in duplicate ($n = 2$)

profiles, for sodium alginate microparticles obtained in aqueous solution at pH 5.6 and pH 2.0. These two pH values were chosen, trying to simulate different conditions of food products and also stomach conditions. The pH 5.6 (deionized water) was used because it is the most simple solvent and can represent the most common solvent used in the preparation of food products. The pH 2 recreates the pH of acid food formulations and is also a good approximation for the pH of the stomach. Considering the release profiles obtained at pH 2, it was decided to not test additional conditions, namely the enteric conditions, because the microparticles would be completely or partially destroyed before arriving to the intestine.

So, it is observed that sodium alginate microparticles presented a fast release pattern of 90% during the first 20 min, followed by a slower and continuous release. The same pattern was verified for both pH values proving that the release rate of 3-CQA from the sodium alginate-based microparticles is not significantly affected by pH. However, according to Yoo et al. (2006), which used sodium alginate as coating material for the microencapsulation of α-tocopherol by ionic gelation, the in vitro releasing profile was significantly affected by pH. Their results showed that for a simulated gastric fluid (0.05 M HCl with 0.2% NaCl), the sodium alginate microparticle release was less than when exposed to a simulated intestinal fluid (0.05 M phosphate buffer)—28.8% against 81.5%. The same behavior was reported by Kumar and Krishna (2014) for the controlled release of sodium alginate microcapsules of an anti-diabetic drug in three different solutions (pH 1.2, 6.8, and 7.4). They verified an increased drug release at higher pH (pH 7.4 and 6.8) than at lower pH (pH 1.2). Although sodium alginate has been reported as relatively stable at acidic pH (pH 1.2), but easily disintegrated under mild alkali conditions (pH 7.4 and 6.8) (Yoo et al. 2006), the results of our study revealed no interference of pH on the release rate of 3-CQA from sodium alginate-based microparticles.

From Fig. 3, it is also possible to estimate the microencapsulation efficiency that considers the amount of 3-CQA that is encapsulated in the time zero. This value is around 60–45% depending on the release profile (pH 2 or pH 5.6) used to do this estimation. This result has always a significant error associated because the release is very fast and it is not possible to measure with accuracy the real-time zero.

Regarding the release profile of 3-CQA from microcapsules performed with modified chitosan at two different pH values (pH 5.6 and 2.0), a slower release was observed (Fig. 4) and the estimated microencapsulation efficiency was around 60%.

Modified chitosan microcapsules presented a release pattern of 85% during the first 1 h, followed by a continuous release. In this case, the complete release of 3-CQA was achieved after 4 h and the same pattern was verified for both pH values.

As for sodium alginate-based microparticles, the obtained release rate of chlorogenic acid from the modified chitosan microparticles was not significantly affected by pH. The 3-CQA microparticles were prepared using different water-soluble biopolymers and by spray-drying method that, as mentioned before, produces normally matrix-type microparticles. The release mechanisms are controlled by solvent action

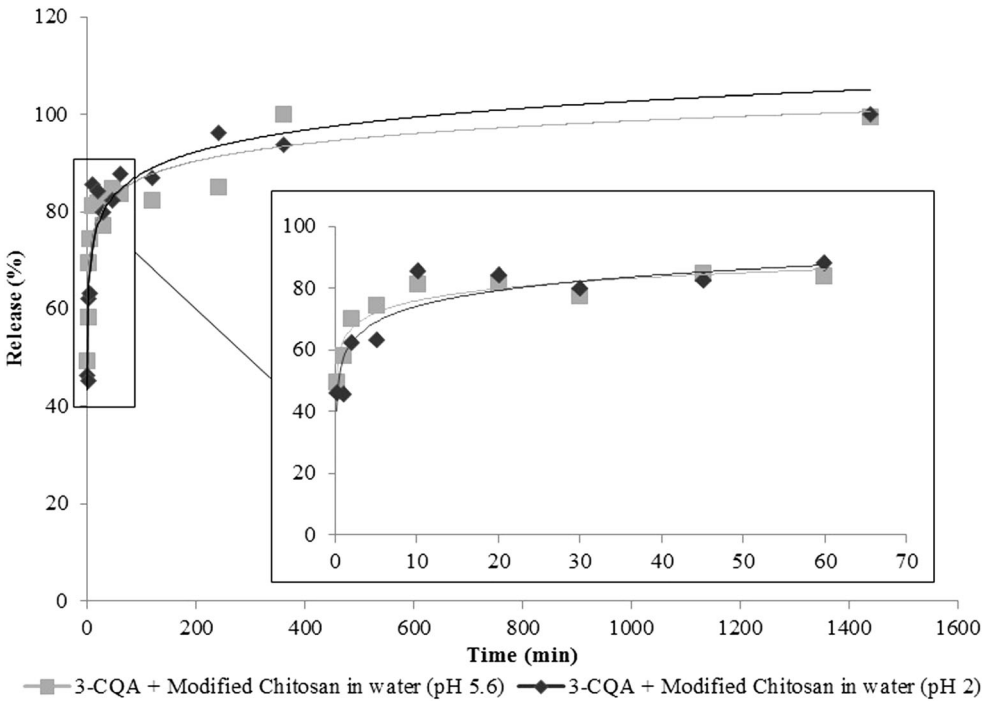

Fig. 4 Chlorogenic acid (3-CQA) release profile from modified chitosan microparticles in distilled water at two different pH values (pH 5.6 and pH 2.0). The assays were done in duplicate ($n = 2$)

and by diffusion. All these factors can be associated to a fast release of the chlorogenic acid.

Other authors reported different release profiles using different wall materials. Nallamuthu et al. (2014) reported the release profile of chlorogenic acid from microparticles prepared with chitosan and the same behavior as described by Yoo et al. (2006) was obtained. The release of chlorogenic acid was faster at neutral pH (PBS, pH 7.4) than at lower pH (HCl, pH 2). Shi et al. (2007) reported different chlorogenic acid release profiles from yeast-encapsulated cells in experiments at different pH values: the release rate of chlorogenic acid in simulated gastric fluid (HCl, pH 1.2) was higher than those obtained in distilled water and in intestine conditions (PBS, pH 7.4). These differences confirm the significant influence of the wall material in the release profiles.

Although the chlorogenic acid microparticles produced in this work had a relatively fast release profiles, the microencapsulation was successfully achieved, allowing the protection from oxidation until the moment of their administration, increasing, consequently, their shelf life.

Antioxidant Activity

Antioxidant activity for 3-CQA microparticles, free 3-CQA, and both encapsulating agents is presented in Table 2. According to the results, 3-CQA microparticles, sodium alginate, and modified chitosan biopolymers presented significant values of antioxidant activity. Modified chitosan showed the highest antioxidant activity (217 ± 16 μM TE), while the value for sodium alginate was 129 ± 1 μM TE, proving to be the best option as encapsulating agent for enhanced antioxidant activity of chlorogenic acid microparticles. Comparing the antioxidant activity of free 3-CQA (151 ± 5 μM TE) with the values obtained for sodium alginate and modified chitosan-loaded microparticles (279 ± 2 and 240 ± 16 μM TE, respectively), it is possible to observe that microencapsulation increases significantly the antioxidant property of 3-CQA. The microencapsulated formulation presents an increase of the antioxidant activity when compared with the free formulation of 3-CQA, in part due to the contribution of the antioxidant activity of the biopolymers.

Table 2 Antioxidant activity of the produced microparticles, free 3-CQA, and modified chitosan and sodium alginate biopolymers. The assays were done in duplicate ($n = 2$)

Analyzed sample	Antioxidant activity (μM TE)
3-CQA microparticles prepared with modified chitosan	279 ± 2
3-CQA microparticles prepared with sodium alginate	240 ± 16
3-CQA	151 ± 5
Modified chitosan	217 ± 16
Sodium alginate	129 ± 1

Moisture Content

Moisture content is an important and normally required parameter in food analysis since it is extensively related with the stability, quality, and composition of food products.

The obtained results show low moisture content for both of the analyzed samples: 3.75% for sodium alginate and 4.52% for modified chitosan microparticles. The values of the moisture content are normal for this kind of microencapsulation process, and they are in the range considered regular and acceptable for food products. These low values prove the stability of the microparticles since that lower moisture content prevents damaging interactions with external factors, such as hosting of microorganisms. For industrial applications, the low moisture content presented by the produced microparticles can be seen as a beneficial support in the enhancement of products' shelf life during their storage or food processing.

In conclusion, the microencapsulation is a general way to increase the stability of the core compounds and this can be observed in the results. These two encapsulating agents (modified chitosan and sodium alginate) increased the antioxidant property and stability of the microencapsulated formulations containing 3-CQA, when compared with the free form. Future works can be developed to study the antioxidant activity and stability during the storage and how different parameters can affect the bioavailability and the stability of the microparticles.

These microparticles can be used with health advantages, for example, in drinks or gelatines prepared instantaneously from powder formulations. Thus, the CQAs will be protected from oxidation, light, moisture, and other factors during the storage time. These are immediate applications of the microencapsulated CQA formulations. However, the incorporation of these microparticles in other types of food products will require additional stability studies, and they will need to be designed considering the type of food product pretended.

Conclusions

Sodium alginate and modified chitosan-loaded microparticles of chlorogenic acid (3-CQA) were prepared by spray drying in order to enhance its stability and bioavailability for food applications. The results prove that 3-CQA was successfully encapsulated into sodium alginate and modified chitosan using spray-drying technique and a satisfactory product yield was obtained for encapsulation in both wall materials (41.1 and 39.3% for sodium alginate and modified chitosan, respectively).

The produced microparticles, with both encapsulating agents, presented an average particle size (volume distribution) around 3 μm. All the microparticles revealed a spherical shape and smooth surface, but chitosan microparticles showed a more regular morphology.

Comparing the results obtained for the release profiles, a slower release was obtained for modified chitosan microparticles. Furthermore, it was also possible to verify that the release rate of 3-CQA was not significantly affected by pH changes.

Antioxidant assessment shows that microencapsulation process increased significantly the antioxidant activity of 3-CQA (279 ± 2 and 240 ± 16 μM TE) when compared with its free form (151 ± 5 μM TE). Although both encapsulating agents presented a positive response for antioxidant activity, modified chitosan showed a higher antioxidant activity value than sodium alginate biopolymer proving to be a better option for enhanced antioxidant activity of 3-CQA microparticles.

Low values of moisture content prove the stability of the microparticles and their beneficial support in the enhancement of products' shelf life namely for food industry applications.

The present work strongly suggests that microparticles prepared by spray drying using sodium alginate and modified chitosan as encapsulating agents can be used for food and health applications of 3-CQA.

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References

Ayelign, A., & Sabally, K. (2013). Determination of chlorogenic acids (CGA) in coffee beans using HPLC. *American Journal of Research Communication, 1*(2), 78–91.

de Azeredo, H. M. C. (2005). Encapsulação: aplicação à tecnologia de alimentos. Alim. Nutr. Araraquara, 89–97.

Belay, A., & Gholap, A. V. (2009). Characterization and determination of chlorogenic acids (CGA) in coffee beans by UV-Vis spectroscopy. *African Journal of Pure and Applied Chemistry, 3*(11), 234–240. doi:10.5897/AJPAC.

Bradley Jr., R. L. (2010). Moisture and total solids analysis. In *Food Analysis* (pp. 85–215) doi:10.1007/978-1-4419-1478-1_6

Cano-Marquina, A., Tarín, J. J., & Cano, A. (2013). The impact of coffee on health. *Maturitas, 75*(1), 7–21. doi:10.1016/j.maturitas.2013.02.002.

Carvalho, I. T., Estevinho, B. N., & Santos, L. (2015). Application of microencapsulated essential oils in cosmetic and personal health care products—a review. *International Journal of Cosmetic Science, n/a-n/a.* doi:10.1111/ics.12232.

Casanova, F., Estevinho, B. N., & Santos, L. (2016). Preliminary studies of rosmarinic acid microencapsulation with chitosan and modified chitosan for topical delivery. *Powder Technology, 297*, 44–49. doi:10.1016/j.powtec.2016.04.014.

Đorđević, V., Balanč, B., Belščak-Cvitanović, A., Lević, S., Trifković, K., Kalušević, A., et al. (2014). Trends in encapsulation technologies for delivery of food bioactive compounds. *Food Engineering Reviews.* doi:10.1007/s12393-014-9106-7.

Duarte, G. S., Pereira, A. A., & Farah, A. (2010). Chlorogenic acids and other relevant compounds in Brazilian coffees processed by semi-dry and wet post-harvesting methods. *Food Chemistry, 118*(3), 851–855. doi:10.1016/j.foodchem.2009.05.042.

Estevinho, B. M. A. N., Rocha, F. A. N., Santos, L. M. D. S., & Alves, M. A. C. (2013a). Using water-soluble chitosan for flavour microencapsulation in food industry. *Journal of Microencapsulation, 30*(6), 571–579. doi:10.3109/02652048.2013.764939.

Estevinho, B. N., Rocha, F., Santos, L., & Alves, A. (2013b). Microencapsulation with chitosan by spray drying for industry applications—a review. *Trends in Food Science and Technology, 31*(2), 138–155. doi:10.1016/j.tifs.2013.04.001.

Estevinho, B. N., Damas, A. M., Martins, P., & Rocha, F. (2014a). Microencapsulation of β-galactosidase with different biopolymers by a spray-drying process. *Food Research International, 64*, 134–140. doi:10.1016/j.foodres.2014.05.057.

Estevinho, B. N., Damas, A. M., Martins, P., & Rocha, F. (2014b). The influence of microencapsulation with a modified chitosan (water soluble) on β-galactosidase activity. *Drying Technology, 32*, 1575–1586. doi:10.1080/07373937.2014.909843.

Estevinho, B. N., Ramos, I., & Rocha, F. (2015). Effect of the pH in the formation of β-galactosidase microparticles produced by a spray-drying process. *International Journal of Biological Macromolecules, 78*, 238–242. doi:10.1016/j.ijbiomac.2015.03.049.

Estevinho, B. N., & Rocha, F. (2016). Microencapsulation in food biotechnology by a spray-drying process. In Ravishankar Rai V. (Ed.), *Advances in Food Biotechnology* (pp. 593–606). John Wiley & Sons, Ltd. doi:10.1002/9781118864463.ch36

Estevinho, B. N., Carlan, I., Blaga, A., & Rocha, F. (2016). Soluble vitamins (vitamin B12 and vitamin C) microencapsulated with different biopolymers by a spray drying process. *Powder Technology, 289*, 71–78. doi:10.1016/j.powtec.2015.11.019.

Fang, Z., & Bhandari, B. (2010). Encapsulation of polyphenols—a review. *Trends in Food Science & Technology, 21*(10), 510–523. doi:10.1016/j.tifs.2010.08.003.

Farah, A., & Donangelo, C. M. (2006). Phenolic compounds in coffee. *Brazilian Journal of Plant Physiology, 18*(1), 23–36. doi:10.1590/S1677-04202006000100003.

Farah, A., & Duarte, G. (2015). Bioavailability and metabolism of chlorogenic acids from coffee. In *Coffee in Health and Disease Prevention* (pp. 789–801).

Farah A, Monteiro M, Donangelo CM, Lafay S (2008). 5-O-caffeoylquinic acid (5-CQA) from green coffee extract are highly bioavailable in humans. *Journal of Nutrition,* (September), 2309–2315. doi:10.3945/jn.108.095554.Federal

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: an overview. *Food Research International, 40*(9), 1107–1121. doi:10.1016/j.foodres.2007.07.004.

Gonçalves, A., Estevinho, B. N., & Rocha, F. (2016). Microencapsulation of vitamin A: a review. *Trends in Food Science & Technology, 51*, 76–87. doi:10.1016/j.tifs.2016.03.001.

Gonçalves, A., Estevinho, B. N., & Rocha, F. (2017). Design and characterization of controlled-release vitamin A microparticles prepared by a spray-drying process. *Powder Technology, 305*, 411–417. doi:10.1016/j.powtec.2016.10.010.

Komes, D., & Bušić, A. (2014). 3. Antioxidants in coffee. In *Processing and Impact on Antioxidants in Beverages* (pp. 25–32).

Kumar, G. P., & Krishna, L. S. (2014). Formulation and evaluation of Vildagliptin sustained release microspheres. *International Journal of Chemical and Natural Science, 2*(6), 189–194. doi:10.1017/CBO9781107415324.004.

Lorentz, C., Pencreac'H, G., Soultani-Vigneron, S., Rondeau-Mouro, C., De Carvalho, M., Pontoire, B., et al. (2012). Coupling lipophilization and amylose complexation to encapsulate chlorogenic acid. *Carbohydrate Polymers, 90*, 152–158. doi:10.1016/j.carbpol.2012.05.008.

Moeenfard, M., Rocha, L., & Alves, A. (2014). Quantification of caffeoylquinic acids in coffee brews by HPLC-DAD. *Journal of Analytical Methods in Chemistry, 2014*. doi:10.1155/2014/965353.

Monteiro, M. C., & Farah, A. (2012). Chlorogenic acids in Brazilian *Coffea arabica* cultivars from various consecutive crops. *Food Chemistry, 134*(1), 611–614. doi:10.1016/j.foodchem.2012.02.118.

Munin, A., & Edwards-Lévy, F. (2011). Encapsulation of natural polyphenolic compounds; a review. *Pharmaceutics, 3*(4), 793–829. doi:10.3390/pharmaceutics3040793.

Nallamuthu, I., Devi, A., & Khanum, F. (2014). Chlorogenic acid loaded chitosan nanoparticles with sustained release property, retained antioxidant activity and enhanced bioavailability. *Asian Journal of Pharmaceutical Sciences, 10*(3), 203–211. doi:10.1016/j.ajps.2014.09.005.

Nesterenko, A., Alric, I., Silvestre, F., & Durrieu, V. (2013). Vegetable proteins in microencapsulation: a review of recent interventions and their effectiveness. *Industrial Crops and Products, 42*, 469–479. doi:10.1016/j.indcrop.2012.06.035.

Niseteo, T., Komes, D., Belščak-Cvitanović, A., Horžić, D., & Budeč, M. (2012). Bioactive composition and antioxidant potential of different commonly consumed coffee brews affected by their preparation technique and milk addition. *Food Chemistry, 134*(4), 1870–1877. doi:10.1016/j.foodchem.2012.03.095.

Perrone, D., Farah, A., Donangelo, C. M., de Paulis, T., & Martin, P. R. (2008). Comprehensive analysis of major and minor chlorogenic acids and lactones in economically relevant Brazilian coffee cultivars. *Food Chemistry, 106*(2), 859–867. doi:10.1016/j.foodchem.2007.06.053.

Qi, L., Shao-qian, C., Si-yi, L., Ying, Z., Li, G., & Si-yi, P. (2010). Microencapsulation of chlorogenic acid in yeast cells. *Food Science, 31*(10), 137–141.

Ramírez-Ambrosi, M., Caldera, F., Trotta, F., Berrueta, L. Á., & Gallo, B. (2014). Encapsulation of apple polyphenols in β-CD nanosponges. *Journal of Inclusion Phenomena and Macrocyclic Chemistry, 80*(1), 85–92. doi:10.1155/2013/251754.

Sato, Y., Itagaki, S., Kurokawa, T., Ogura, J., Kobayashi, M., Hirano, T., et al. (2011). In vitro and in vivo antioxidant properties of chlorogenic acid and caffeic acid. *International Journal of Pharmaceutics, 403*(1–2), 136–138. doi:10.1016/j.ijpharm.2010.09.035.

Shi, G., Rao, L., Yu, H., Xiang, H., Pen, G., Long, S., & Yang, C. (2007). Yeast-cell-based microencapsulation of chlorogenic acid as a water-soluble antioxidant. *Journal of Food Engineering, 80*(4), 1060–1067. doi:10.1016/j.jfoodeng.2006.06.038.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis, 19*(6–7), 669–675. doi:10.1016/j.jfca.2006.01.003.

De Vos, P., Faas, M. M., Spasojevic, M., & Sikkema, J. (2010). Encapsulation for preservation of functionality and targeted delivery of bioactive food components. *International Dairy Journal, 20*(4), 292–302. doi:10.1016/j.idairyj.2009.11.008.

Yoo, S.-H., Song, Y.-B., Chang, P.-S., & Lee, H. G. (2006). Microencapsulation of alpha-tocopherol using sodium alginate and its controlled release properties. *International Journal of Biological Macromolecules, 38*(1), 25–30. doi:10.1016/j.ijbiomac.2005.12.013.