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EFFECT OF CROSSLINKING AND ATOMIZATION PARAMETERS ON THE PROPERTIES OF ALGINATE MICROPARTICLES FORMED BY IONIC GELATION

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Abstract: Sodium alginate is a non-toxic and biodegradable polysaccharide with high gelling capacity that makes it an excellent material for the development of delivery systems for drugs and bioactive compounds and various environmental applications. In this study, alginate microparticles were prepared through ionic gelation by atomization, varying the concentration of the $CaCl_2$ bath (3 and 6% m/m), air pressure (0.25 and 0.50 bar) and spraying distance (20 and 25 cm). The microparticles obtained were evaluated for size distribution, surface morphology and stability against different pHs (pH 2.5, 3.5 and 4.5) for 4 days. The atomization process produced particles with sizes between 83 and 146 μm with different morphologies (irregular, spherical or elliptical). It was observed that the particles produced with 0.25 bar underwent changes in size and morphology after 4 days depending on the pH of the medium. Samples produced at a concentration of 6% $CaCl_2$ showed larger sizes when compared to those formed with 3%, possibly due to the greater ionic strength of the solution. As for the spraying height, the samples produced at 25cm had a more spherical morphology than the others. These results allow the development of microparticles with desirable properties from the control of the conditions of the atomization and reticulation process.

INTRODUCTION

Sodium alginate is a polysaccharide extracted from brown algae, biocompatible, biodegradable, non-toxic and with high gelling capacity. Gel formation is commonly induced by ionic gelation especially in the presence of divalent ions such as calcium(Ca^{2+}). Its structure composed of copolymers of α-L-glucuronic acid (G) and β-D-mannuronic acid (M) (Soliman et al., 2013) allows the establishment of salt bridges with calcium, originating a cross structure called "egg box" (Zhang et al., 2021).

Ionic gelation is an encapsulation technique that allows the trapping of bioactive compounds and microorganisms with the aim to increase resistance and stability to external environments, and which has the advantages of low cost, simplicity and absence of organic solvents. The particles can be formed by dripping or atomization, allowing the control of their characteristics and size according to the process conditions employed (de Moura et al., 2022). Larger particles formed by dripping generally offer greater protection to the bioactive incorporated inside them due to the smaller area/volume ratio of the particle. However, the uniform dispersion of particles in fluid matrices is compromised (de Moura et al., 2018), in addition to being perceptible to touch and taste, in the case of food applications, which limits their size to values smaller than 100 μm (Leong et al., 2016). In addition, the size and morphology of the formed particles directly influence their stability against adverse environmental conditions, as well as the release profile of bioactive in food, cosmetics and along the gastrointestinal tract. Such characteristics are strongly dependent on the conditions of atomization and crosslinking of alginate gels.

Therefore, the aim of the present study was to evaluate the effect of atomization conditions and crosslinker concentration on the properties of calcium alginate particles. Two concentrations of $CaCl_2$ were evaluated in order to change the availability of Ca^{2+} ions for alginate crosslinking (Soliman et al., 2013), air pressure and spraying distance. The microparticles were evaluated for size and morphology, as well as stability over time against different pH values.

MATERIAL AND METHODS

MATERIAL

The materials used in this work were

Grindsted® Alginate FD 175 sodium alginate (France), kindly provided by Danisco. The other reagents used were of analytical grade.

METHODS

Formation of microparticles: Sodium alginate 1.5% (m/m) was dissolved in ultrapure water (Milli-Q) under magnetic stirring at 420 rpm for 1.5 hours at 30 ºC. The alginate solution (1.5% (m/m)) was atomized in a UM-10 atomizing nozzle with a diameter of 1.0 mm at room temperature (25 ºC). The spraying distance, $CaCl_2$ solution concentration and air pressure were varied according to Table 1. The system flow at 0.25 and 0.50 bar was 3.35 and 5.60 mL/min, respectively. After atomization, the particles remained for 15 minutes in $CaCl_2$ solutions under mild magnetic stirring. Then, the particles were vacuum filtered and washed with distilled water. Part of the particles were incubated at different pHs to assess stability over time. The other fraction was analyzed for size and morphology at post-preparation. The yield was determined according to Equation 1:

$$Yield\ (\%) = \frac{Mf}{Mi} \times 100 \qquad (1)$$

where Mf is the final mass of microparticles and Mi is the initial mass of the alginate solution.

CHARACTERIZATION OF PARTICLES

Particle Size: To evaluate the particle size, static light scattering was determined in *Mastersizer* (*Malvern Instruments* Ltd, Marvern, Reino Unido). The particles were dispersed in distilled water for reading in 6 repetitions. The results were expressed as volumetric mean diameter (De Brouckere diameter – $D_{[4,3]}$) (Equation 2). The median value of size distributions was evaluated (D_{50}).

$$D_{[4,3]} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \qquad (2)$$

where, d_i is the particle diameter and n_i is the number of particles.

The polydispersion index (PDI) was calculated according to de Moura et al. (2019) using Equation 3.

$$PDI = \frac{D_{90} - D_{10}}{D_{50}} \qquad (3)$$

where D_{90} e D_{10} are the diameters of 90% and 10% of the accumulated volume, respectively.

Morphology: Particle morphology was examined by light microscopy using a model JENAVAL 30-G0020 microscope (Carl Zeiss, Gottingen, Germany).

Stability: To evaluate the stability of the microparticles at different pHs, they were incubated at a 1:10 ratio (microparticle:buffer) in 0.2 mol/L glycine buffer pH 2.5 and 0.2 mol/L sodium acetate buffer in pH 3.5 and pH 4.5 for 4 days. After this period, the particles were evaluated for size and morphology.

Statistic: Analyzes were performed in triplicate. The difference between means was verified by ANOVA and Tukey's test ($p<0.05$) using the PAST *software* (HAMMER; HARPER; RYAN, 2001).

RESULTS

The atomization method used for calcium alginate particle formation has the driving force the air at high speed that passes through a small nozzle causing the aspiration of the sodium alginate solution, through the venturi effect (Smyth et al., 2011). Thus, the sodium alginate solution is sprayed onto the crosslinking solution of divalent cations (Ca^{2+}) resulting in an insoluble three-dimensional network structure. The present study produced small-sized microparticles (between 83 and 146 μm) and varied shapes, in agreement with what was reported by Ching et al., (2017).

The best yield was observed in the sample with lower pressure (0.25 bar) (Table 1) with

greater height and lower concentration of crosslinking agent. In general, lower yields were observed for particles formed by 3% (m/m) of $CaCl_2$, which may be associated with greater water retention in such samples. At higher ionic strengths, the density of the alginate network is greater due to the large amount of salt bridges. This can decrease your systems water holding capacity, causing particles to shrink. As the yield calculation was carried out on a wet basis, the amount of water retained by the particles contributed to the observation of higher yield values.

Particle Size:

The particle size characterizations are presented in Table 1. For all samples, monomodal distributions were found with high PDI values (3.12 to 5.79), an expected result due to the atomization providing the chaotic rupture of the alginate solution under conditions turbulent (Leong et al., 2016).

Sample	Pressure (bar)	Height (cm)	[$CaCl_2$] (% m/m)	Yield (%)	$D_{[4,3]}$ µm	D_{50} µm	PDI
0.25-20-3	0.25	20	3	11.85^{d}	146.29 ± 13.19^{a}	68.79 ± 3.98^{a}	5.79 ± 0.32^{a}
0.25-20-6	0.25	20	6	18.84^{b}	97.55 ± 3.63^{c}	54.98 ± 1.47^{b}	4.01 ± 0.12^{de}
0.25-25-3	0.25	25	3	20.71^{a}	83.06 ± 2.51^{d}	40.79 ± 0.35^{f}	4.97 ± 0.32^{b}
0.25-25-6	0.25	25	6	11.72^{d}	89.03 ± 0.33^{d}	51.38 ± 0.56^{d}	3.77 ± 0.05^{df}
0.50-20-3	0.50	20	3	13.96^{c}	85.49 ± 4.81^{d}	50.88 ± 0.82^{d}	3.47 ± 0.30^{d}
0.50-20-6	0.50	20	6	13.15^{cd}	91.18 ± 8.41^{cd}	$51.89\ 2 \pm 2.15^{bcd}$	4.03 ± 0.42^{d}
0.50-25-3	0.50	25	3	13.40^{cd}	84.20 ± 3.20^{d}	53.98 ± 0.69^{bc}	3.12 ± 0.20^{dg}
0.50-25-6	0.50	25	6	9.85^{e}	138.15 ± 6.78^{ab}	70.45 ± 1.97^{ae}	4.86 ± 0.19^{bc}

Different letters in the same column indicate a significant difference $p < 0,05$

Table 1. $CaCl_2$ concentrations, spray height and air pressure used in particle formation; process yield; De Brouckere diameter ($D_{4.3}$), median of particle size distributions and polydispersion index (PDI).

Spray distance was a relevant factor for particle size (Table 1). There was a significant decrease in all size parameters analyzed ($D_{[4,3]}$, D_{50} e PDI) of samples atomized at 0.25 bar and 25 cm compared to 20 cm. The greater distance allowed for a more homogeneous formation of the drops before touching the surface of the crosslinking solution, resulting in more uniform and smaller particles.

On the other hand, the samples produced at 0.50 bar did not show a significant difference when the spray height was changed, however, a slight decrease in the size of the $D_{[4,3]}$ (except for 0.5-0.25-6, in which aggregation was observed on microscopy (data not shown)).

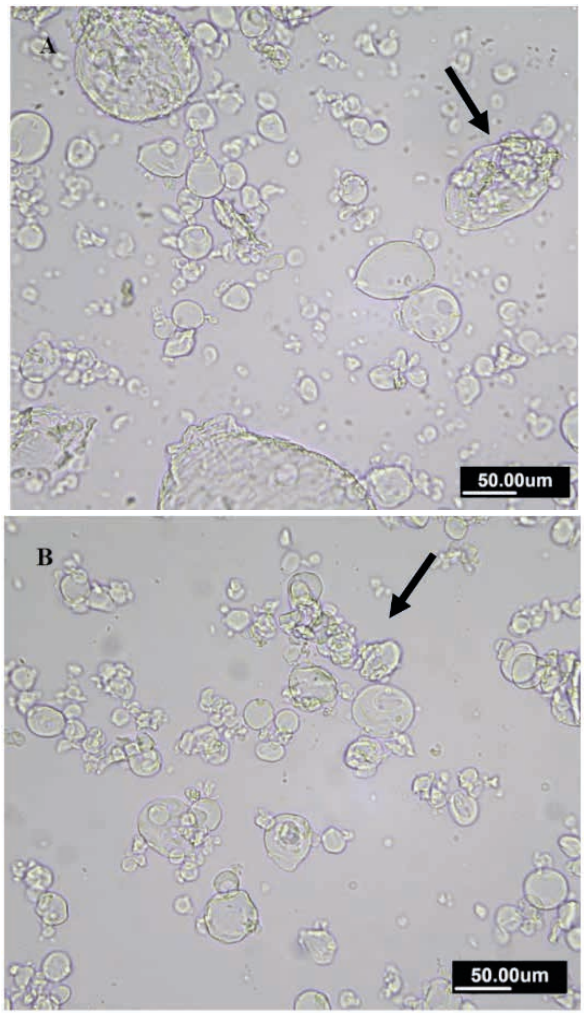
In general, it is observed that the particle size decreases with increasing pressure, due to the speed of breaking the surface tension between the alginate solution flow and the air during droplet formation (Smrdel et al., 2008). In addition, such samples presented significantly lower PDI, especially for the samples of 3% (m/m) of $CaCl_2$, indicating greater homogeneity of the particles in relation to size.

Morphology: Freshly prepared microparticles were analyzed for morphology (Figure 1). According to Hariyadi et al., (2010), due to the impact of the alginate solution droplets with the reticulation solution, it is expected that the microparticles have an irregular elliptical shape.

For the particles formed in a 3% (m/m) $CaCl_2$ bath, the samples produced with lower air pressure (0.25 bar) showed irregular morphology, greater aggregation and fractured

particles. This pressure may not have been enough to break the surface tension between the alginate solution flow and the air during droplet formation (Perrechil et al., 2012).

At 0.50 bar, greater sphericity is observed, especially for particles 0.5-25-3, in addition to greater sample homogeneity, which is reflected in the lower PDI values (Table 1).



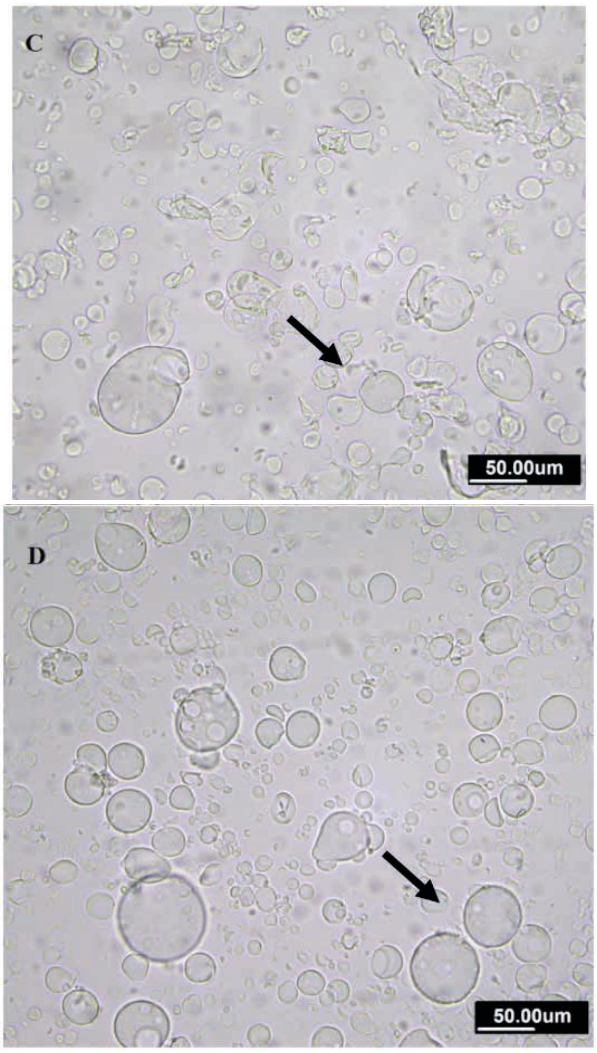


Figure1: Sample morphology.A) 0.25-20-3, B) 0.25-25-3, C) 0.5-20-3, D) 0.5-25-3

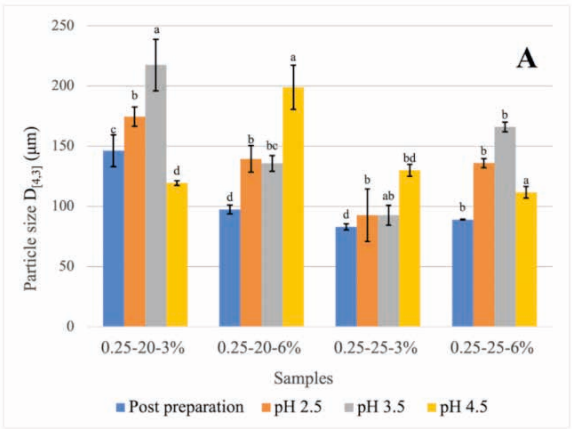
Stability: After preparation, the microparticles were incubated at pH 2.5, pH 3.5 and pH 4.5 to evaluate stability against different pH values. In Figure 2, it is possible to compare the size of the microparticles immediately after preparation and after 4 days of storage.

Alginate presents pKa 3.4, and its structure has the presence of carboxylic groups that confer great sensitivity to external pH stimuli. pH values below the pKa promote insolubility of the alginate network due to the non-ionized form of the carboxyl groups (COOH). However, for pHs greater than 3.4, the carboxylic groups become ionizable (COO-),

increasing the electrostatic repulsion between the negative charges and may promote swelling of the hydrophilic matrix (Agüero et al., 2017).

Samples produced at 0.25 bar showed a statistically significant difference in size at different pHs, with the 0.25-25-6 sample being the most stable, showing variation at pH 4.5. When the air pressure is increased to 0.50 bar the samples tend to show greater stability, especially at 0.5-20-6, which did not show statistically significant size variation. Both samples mentioned as more stable were produced in a 6% (m/m) CaCl2 bath. According to Dodero et al. (2019) particles formed with high crosslinking density have low water absorption due to the greater number of restrictions between the polymer chains. In addition, the increased reticulation also causes greater shrinkage of the microparticles, reducing the porosity of the network and propensity for swelling.

The restriction of the polymeric chains and consequent low water absorption prevented the ionic exchange of these samples, unlike the others in which ionic exchange between the ions may have occurred Na^{+} present in the buffer solutions (pH 3.5 and 4.5) with the ions Ca^{2+}, representing the initial phase of the gel swelling process (Bajpai & Sharma, 2004).



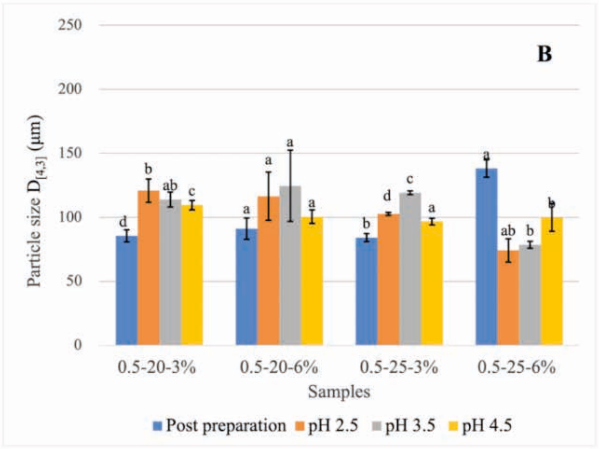


Figure2. Size stability of samples produced at different atomization pressures when stored at different pH values (2.5, 3.5 and 4.5) for 4 days at 10 °C. A) 0.25 bar B) 0.50 bar. Sample sizes right after preparation were considered Control. Different letters in the same dataset (by sample) indicate a significant difference $p<0.05$.

CONCLUSION

The height and pressure of atomization and the concentration of the crosslinking bath strongly influenced the yield of the process, the size and morphology of the particles. Although the atomization at 0.25 bar presents better performance, the stability of the particles formed in this condition against acidic pHs was lower. On the other hand, the yield of the process at 0.5 bar was lower, but the particles were more spherical and stable at the different evaluated pHs, which may be interesting for applications in food and for the delivery of bioactives.

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