Microencapsulation of Grapefruit Oil with Sodium Alginate by Gelation and Ionic Extrusion: Optimization and Modeling of Crosslinking and Study of Controlled Release Kinetics.

Microencapsulación de aceite de pomelo con alginato de sodio por gelificación y extrusión iónica: optimización y modelado de la reticulación y estudio de la cinética de liberación controlada.

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Abstract

Essential grapefruit oil with high concentrations of limonene is used in food, cosmetic and pharmaceutical industries due to its antimicrobial properties, fragrance, and flavour. To facilitate its manipulation and protect it from adverse environmental factors, the microencapsulation is used. The objective of this work was to optimize the microencapsulation process of grapefruit essential oil using external ionic gelation coupled to extrusion with sodium alginate and calcium chloride. We achieved the best encapsulation conditions with calcium chloride concentration at 7.4% w/v and a crosslinking time of 58 minutes, obtaining a yield of 62% and an efficiency of 100% with an oil loading capacity of 10% w/w. The chemical adsorption of calcium as well during the crosslinking process was studied, observing a significant fit with the Elovich equation. And an adjustment of the controlled release of the essential oil was obtained to the empirical kinetic model of Korsmeyer and Peppas.

Keywords: sodium alginate, encapsulation, essential oils, ionic gelation, crosslinking.

Introduction

Grapefruit oil is an essential oil (EO) known as a sub product of the citrus industry, of great interest for its antimicrobial properties. It is a growth inhibitor of Escherichia coli, Staphylococcus aureus, Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. diacetylactis, Leuconostoc mesenteroides subsp. dextranicum and Lactobacillus plantarum (Vasek, et al., 2015; Ribeiro Santos et al., 2017), making it appropriate for the food and pharmaceutical industry.

Furthermore, this type of oil contains a high percentage of limonene (higher than 70%), which makes it attractive for the cosmetic and cleaning industries, and also applicable as a food additive and industrial solvent as well (Bakkali, et al., 2008). Citrus essential oils represent 0.27% of the total export from Argentina, so it could be considered as a product with great potential for industrial development (Federcitrus, 2018).

Industrial applications of EO are limited by their high degree of volatility, a characteristic that significantly affects the yield of these processes (Soliman et al., 2013). Furthermore, their sensibility to external agents like ultraviolet light, high temperatures, and water, may
Affect their composition, which facilitates the loss of some specific properties (Lv et al., 2012). As a matter of fact, it is necessary to develop a system that may protect them and facilitate their manipulation (Chen et al., 2017; Kuang et al., 2010).

Microencapsulation is a coating technique which may be used as a system of protection of the agent or coating component, to protect them from external factors. In the case of EO, it also allows small dosages (Benavidez, Cortés, Parada and Franco, 2016; Aghbashlo et al., 2012).

In recent years, there has been a growing interest in microencapsulation of EO with biodegradable materials. Applications were reported in the pharmaceutical industry (Balanc et al., 2016; Goh et al., 2012; Liakos et al., 2014; Liu et al., 2012), in the nutraceutical industry (Mokhtari et al., 2017) and in the food industry (Arriola et al., 2019; Laokuldilok et al., 2016; Lupo et al., 2015) for the immobilization of enzymes or bacteria in bioprocesses. (Ding and Shah, 2007; Shory, 2017; Aguiar et al., 2016).

Several methods of EO microencapsulation have been described for different authors, such as spray drying (Baranauskiene et al., 2006; Aghbasho et al., 2012; Tuyen et al., 2014), coacervation (Dima et al., 2014; Jun-Xia et al., 2011) and ionic gelation (Chan et al., 2000; Liakos et al., 2014; Hosseini et al., 2013).

Ionic gelation by extrusion is an encapsulation technique which presents great adaptability to different types of active principles, whether hydrophobes or hydrophilics, and is a low-cost procedure (Arriola et al., 2019).

Optimization of EO microencapsulation with alginate has been currently studied for its implementation in the food industry considering its antimicrobial properties, smell and taste (Noppakundilograt et al., 2015; Soliman et al., 2013; Goitia Funes and Amurrio Derpic, 2016). Furthermore, sodium alginate is one of the polymeric materials widely used as a matrix to encapsulate active principles due to its biocompatibility, biodegradability and inherent nature (Hosseini et al., 2013; Chuah et al., 2009). It is composed of β-D-mannuronic and α-L-guluronic units linked by 1-4 β-(1–4) guluronic acid units (Liakos et al., 2014; Levic et al., 2016). The alginate has the capacity of producing ionically cross-linked hydrogels, known as the “egg-box model” and generally accepted as the gelation mechanism (Lertsutthiwong et al., 2008). The crosslinking mechanism involves an intermolecular union between divalent cations (for example, calcium) and carboxyl groups in the residues of the guluronic acid of linear polymer chains (Belscak-Cvitanovic et al., 2016). The concentration of calcium chloride solution and the crosslinking reaction have an effect on the efficiency and yield during the encapsulation process. The matrix obtained after crosslinking can lead to a controlled release (Bal et al., 2013) with a release mechanism of the active components sustained in time (Finch, 2005).

In this study, we optimized the microencapsulation process of the grapefruit essential oil, a regional product with a high percentage of limonene, through external ionic gelation with sodium alginate by extrusion. We applied a two-factor experimental design with three levels in order to obtain a high concentration of EO. The concentration of calcium chloride and the crosslinking time were analyzed in terms of yield and efficiency of microencapsulation. We analyzed the controlled release as well, and the characteristics of the microcapsules.

Material and Methods

Material

It was employed a sodium alginate solution (Aldrich with 39% L-guluronic acid and 61% of mannuronic acid, molecular weight of 120,000-190,000 g/mol) and anhydrous calcium chloride.
chloride (Cicarelli). Grapefruit essential oil was provided by Mager Enterprise (from the province of Corrientes, in the northeast of Argentina).

**Chemical constitution of grapefruit essential oil**

Identification of grapefruit EO components were conducted by gas chromatography-mass spectrometry (GC/MS). A two-capillary column QP 5050 Shimadzu equipment was used: one SE 52 (Mega, Legnano, Italia) chemically bonded (25 m x 0.25 mm internal diameter; 0.25 µm thick stationary phase) column, covered by 5% phenil-polydimethylsiloxane (0.25 µm thick stationary phase) at a column temperature of 60 ºC (8 min), increasing up to 180 ºC at a 3 ºC/min speed, then up to 230 ºC at a 20 ºC/min speed. 250 ºC injector temperature, split injection mode; 1:40 split ratio; 0.2 µl oil injection volume. Mobile phase: Helium, 122.2k Pa (51.6 cm/sec), 250 ºC interface temperature and 40-400 m/z mass range acquisition. Another BP-20 (SGE, Australia) 25 m x 0.25 mm internal diameter fused silica capillary column covered by polyethyleneglycol 20.000 Da (0.25 µm thick stationary phase). Column temperature at 40 ºC (8 min), increasing up to 180 ºC (3 ºC/min), and up to 230 ºC (20 ºC/min). 250 ºC, injector temperature, split injection mode; 1:40 split ratio; 0.2 µl oil injection volume. Mobile phase: Helium, 92.6 kPa (55.9 cm/s), 250 ºC interface temperature and mass range acquisition: 40-400 m/z. Fragmentation patterns in each component were compared to those stored in the software library spectra (Mc Lafferty and Stauffer, 1991; Adams, 2001).

**Preparation of solutions and alginate-oil emulsion**

A calcium alginate solution was dissolved in deionized water (0.2 µS conductivity, 40 ºC) at a concentration of 1% w/v by soft mechanical stirring. Then, the resulting solution was left standing for an hour in order to de-gas it. After that, the viscosity of the alginate solution was sized with a Ni-Run NDJ-8S digital viscometer using a spindle N° 0. The crosslinking solutions were then prepared with anhydrous calcium chloride in deionized water to 2, 5 and 10% w/v. Separately, 30 g of alginate solution was mixed with the specific amount of grapefruit oil necessary to obtain a 10% w/v load. The DLAB D500 homogenizer used was at 2000 rpm for three minutes to obtain a homogeneous distribution of the emulsion. To assure its stability, the solution was then left standing for an hour, observing if the separation of phases took place (Chan, 2011; Levic et al., 2015).

**Microscopic observation**

The degree of dispersion and homogeneity of the emulsion was determined by measuring the droplet size and calculating the polydispersibility (Mabille et al., 2000). These statements were applied following the Benavides et al. methodology (2016) with slight modifications. Degasification was then performed by putting the emulsion into an ultrasonic bath with a frequency of 40 khz (Digital Ultrasonic Leaner, ARCANO) with water at room temperature (25 ºC) for three minutes.

After that, the emulsion was observed with a trinocular microscope Lancet SME F6E equipped with a camera (Touptek UCMOS 01300 KPA, TP601300A model, origin China) and data acquisition system (Toup View: Touptek Photonics of Touptek Corporation) to size the diameter of the droplets. The polydispersibility of the emulsion was calculated with the following equation (1) (Camino, Sánchez, Patino and Pilosof, 2012):
Statistical analysis: Optimization of the encapsulation process

Experiments were conducted following a 3\textsuperscript{k} central composite factorial design, with k=2, and analyzed according to the response surface methodology to estimate the polynomial quadratic equations of optimization (Kuehl, 2000). Calcium chloride (2, 5 and 10% w/v) and time (30, 45 and 60 minutes) were the factors selected, expecting that both were linked to the yield and efficiency in the obtention of microcapsules. All experiments were performed with a 10% w/w load of essential grapefruit oil and with a sodium alginate polymeric solution to 1% w/v. Two replicates of the design were carried out with nine runs each, fulfilling a total of 18 runs. The statistical analysis was performed with Minitab Statistical Software 18.1 (2017), trial. For this specific design, it was employed the following quadratic model (2):

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \epsilon$$

(2)

Where y is the factor, \(\beta_0\) is the constant, \(\beta_i\) are the mean linear coefficients, \(\beta_{ii}\) are the mean square coefficients, \(\beta_{ij}\) are the mean effect of interactions, \(x_1\) and \(x_2\) are the independent variables, and \(\epsilon\) is the random error (Montgomery, 2003). Analysis of Variance (ANOVA) was then performed with a level of statistical significance of 95% (p<0.05). In addition, the regression coefficient (\(R^2\)), the p-value, the F-value (Fisher Test) and the lack of adjustment were evaluated in order to determine the adjustment of the model. The two responses (yield and efficiency) were maximized. After that, the model was validated with five runs at the optimal point.

Obtention of microcapsules

Microcapsules were obtained following the methodology described by other authors with slight modifications (Chan, 2011; Benavidez et al., 2016). A syringe pump Apema PC11UBT was used to perform extrusion with a needle of internal diameter of 0.21 mm. For this part of the experiment, 10 ml of the emulsion previously prepared were taken with a syringe that was connected in the pump programmed at a dripping speed of 90 mL/h and fixed 5 cm above the surface of the solution. Afterwards, the drops fell into a calcium chloride solution which was prepared according to the design. The gelling solution was gently stirred with a magnetic stirrer over 30, 45 and 60 minutes. The obtained microcapsules were separated from crosslinking with a steel filter net and rinsed into tert-butyl alcohol in order to eliminate oil residues from the surface. Then, the microcapsules were weighed in a balance analyzer and stored in a caramel recipient at 4 °C.

Yield of the encapsulation process

The yield is the rate between the number of microcapsules obtained and the amount of the emulsion used during the encapsulation process. The percentage yield was calculated with the following equation (3) (González et al., 2005):

$$P = \left( \frac{1}{\bar{d}} \right) \left( \frac{\sum (d_i^3 x (\bar{d} - d_i))}{\sum d_i^3} \right)$$

(1)

Where \(d_i\) is the droplet diameter and \(\bar{d}\) is the average droplet diameter of the emulsion.
Efficiency of the microencapsulation process

The efficiency of the microencapsulation process measures the amount of active components that were encapsulated into the microcapsule. The calculation was based on the determination by gas chromatography of the microencapsulated limonene mass (Solomon et al., 2012) using the method of internal standards. In order to measure with a chromatography, the microcapsules were filtered with a steel sieve and rinsed with a considerable known amount of tert-butyl alcohol to remove the residual oil from the surface of the microcapsule. A sample of 30 µL of this solution was taken and then 10 µL of methyl isobutyl ketone were added as internal standard. This mixture was analyzed with a Shimadzu GC–14B gas chromatography, Megabore DB-WAX P/N 125-7032 polar column of 30 m long x 0.53 mm of internal diameter x 1 µ thickness, connected to a Flame Ionization Detector (FID). The FID temperature was of 220 ºC and for the injector temperature was of 180 ºC. The temperature of the column remained isothermal in 40 ºC for over two minutes. Then, it increased to 5 ºC/min until reaching 180 ºC. Lastly, the temperature remained isothermal in 180 ºC for over two minutes. The related areas of the chromatogram in a standard curve were compared in order to determine the limonene mass present on the surface ($w_s$). Afterwards, the weight of the encapsulated limonene was calculated in a specific mass of microcapsules ($w_1$) stating a difference between the weight of the initial ($w_2$) and the superficial ($w_s$) limonene. For this study, the efficiency value was determined as follows (4) (Maji, Baruah, Dube and Hussain, 2007):

$$E\% = \frac{w_1}{w_2} \times 100$$  \hspace{1cm} (4)

Loss of water during the yield process

The moisture of the microcapsules was determined to evaluate the amount of loss of water during the crosslinking process. Almost 0.8 g of filtered microcapsules were weighed and then placed onto an aluminum tray. In order to determine their moisture, it was employed a Radwag PMR 50 moisture balance analyzer and the measurements were assayed in quintuplicate. The microcapsules used were prepared without oil, with 1% w/v alginate crosslinking in calcium chloride solution to 2, 5 and 10% w/v during the crosslinking time of 30, 45 and 60 minutes.

Morphology of the alginate microcapsules

Microcapsules were observed through an optical microscope equipped with a digital camera in order to measure the sphere diameter. The morphology of the microcapsules was evaluated with a Scanning Electron Microscope (SEM) Jeol 5800 LV. Microcapsules were
then placed over the stage and sputtered with a thin coat of gold. Observations were made with different increments at room temperature.

**Crosslinking calcium adsorption**

After the optimization of the process of obtaining microcapsules, the kinetic yield with the optimized values of calcium chloride concentration and the crosslinking time were studied. Then, 1% w/v of alginate solution in deionized water was placed into the syringe pump, without essential oil, and it dripped on the crosslinking solution of calcium chloride 5% w/v. They were cross-linked during 1, 2, 3, 5, 15 and 30 minutes. Microcapsules were then filtered and rinsed in deionized water. Approximately 30 g of microcapsules were weighed and placed into a crystallizer to get dry. After that, they were placed in an oven at 100 °C for over 12 hours. The dried particles were then transferred to a clean and dried crucible and introduced in a muffle furnace at 750 °C for over eight hours or until they turned to ashes. After that process, dried calcium ions were valued by emission spectrophotometry using Jenway PFP07 flame photometer (Industrial Version). The data obtained was precisely revised in order to discover kinetic adsorption. To obtain this, we employed the Elovich model about the application of general processes of chemisorption, using the following equation (5) (Pinzón-Bedoya and Vera Villamizar, 2009):

\[
\frac{dq}{dt} = ae^{-bt^2}.
\]

Where, \(q\) is the adsorbed amount in time \(t\) (mmol/g), \(a\) is the initial speed of adsorption (mol/gmin) and \(b\) is related to the covered surface and the energy of activation by chemisorption (mmol/g).

**Study of the controlled release**

The controlled release was performed by placing 8 g of microcapsules obtained during the optimized process in a 50 mL Erlenmeyer with 20 g of tert-butyl alcohol at room temperature with continuous stirring on an orbital shaker. Aliquots of 30 µL were extracted from the solution at regular intervals of 30 minutes until eight hours of release. Then, a diary sample was taken for two days, and after that, a weekly sample was taken until two weeks were completed. The samples were analysed in a gas chromatograph following the exact conditions described above. The experimental data were presented to make the mathematical model after that.

**Kinetics analysis of drug release**

The mechanism of essential oil release was studied from the matrix to the exterior fitting the data obtained to the semi-empirical model presented as follows (6) (Korsmeyer and Peppas, 1981):

\[
\frac{M_t}{M_\infty} = kt^n
\]

Where \(M_t / M_\infty\) is the fractional solute release, \(t\) is the release time, \(k\) is a constant characteristic of the macromolecular network system and the substance encapsulated, and \(n\) is the diffusional exponent characteristic of the release mechanism.
Results and discussion

Chemical constitution of essential oil

21 components were identified by GC/MS analysis: 9 terpenes, 3 sesquiterpenes, 2 aldehydes, 7 alcohols, and 1 ester (representing 99.10% of the components). The compound present in the highest proportion was limonene (92.6%) near the upper limit of the reported range (76.00-96.00%) for citrus essential oils (Kirbaslar, et al., 2006; Espina, et al., 2011). The complete characterization of the grapefruit essential oil used in this work was carried out in previous studies and published by the authors (Vasek, et al., 2015).

Preparation of solutions and alginate-oil emulsion

The viscosity of the alginate solution at 1% used in the emulsion at 20 °C with a rotor speed of 0.6 rpm was 0.35 kg/ms. This concentration of alginate was established in preliminary studies being ideal for extrusion due to the size of the needle selected. The emulsion prepared with the alginate solution and 10% w/w of grapefruit oil showed stability after an hour of standing, with no phase separation. Levic et al. (2015) reached similar results with grapefruit oil concentrations of 5 and 10% w/v. Chan (2011) demonstrated stability during the same time with oil concentrations from 5 to 40% v/v. Oil drops in the emulsion, observed in the microscope, have an average diameter of 0.0018 mm (Figure 1a), slightly smaller than the diameters obtained by Noppakundilograt et al. (2015) with similar concentrations of alginate emulsion and eucalyptus oil. The polydispersibility calculated with the equation (1) was 0.8, and this value (> 0.5) indicated that it was an emulsion with a broad distribution of diameters. Benavidez et al. (2016) obtained similar polydispersibility values but with lower homogenization speed and less oil concentration, and with a larger average diameter. Additionally, the asymmetry of the sample was calculated and showed a value of 0.36 indicating a positive asymmetry. Thus, it cannot be statistically represented by the sample mean. The mode of the distribution was then calculated, which showed a value of 0.0008 mm. This not-gaussian distribution responded to other distribution models (Sis et al., 2005) and it was a feature of the emulsions prepared at high speeds and with small droplet sizes.

Obtaining of microcapsules

It was possible to obtain spherical microcapsules with a uniform size (Figure 1b) using a dropping distance of 0.05 m and a dropping speed of 90 mL/h (established in preliminary studies).

Fig. 1: Images of (a) optical micrograph (40 × magnification) illustrating the microdroplet sizes of the emulsion prepared with 10% grapefruit essential oil, (b) microcapsules obtained
Microencapsulation yield and efficiency

The levels of the factors and the percentage results of efficiency and yield reached are illustrated in Table 1. The average yields obtained were set between 42% and 68%. These low yield values stemmed from the loss of water during the crosslinking process, which was demonstrated with the moisture analysis of particles after filtration, as it is shown later on Benavides et al. (2016) achieved a maximum yield of 62% using calcium chloride at 1.5% with loading capacity of oil of 1–3% v/v. Other authors (Ribeiro et al., 1999) obtained yields of 60–80% using double emulsions of oil and water, besides incorporating another wall material such as chitosan. In other studies, they obtained yields from 83.4% to 87.97% encapsulating Zanthoxylum limonella essential oil by multiple emulsion solvent evaporation technique.

As it is shown in Table 1, the efficiency of the microencapsulation was related to time. Maximum efficiency was seen at 60 minutes of crosslinking, reaching an average of 99.86% of efficiency in encapsulation. This value could be related to a longer crosslinking time resulting in a wall with a higher level of rigidity, which reduces the retention of essential oil on the surface during gelation. Another study showed lower efficiency (88.6%) at 60 minutes on the encapsulation of eucalyptus essential oil (Noppakundilograt et al., 2015), and observed also dependence with the crosslinking time and independence with the calcium chloride concentration. Soliman et al. (2013) encapsulated cinnamon oil, clove oil, and thyme oil, and found similar efficiency values in encapsulation (between 90 and 95%) using calcium chloride concentrations of 0.5% and crosslinking times of 20 minutes. Efficiencies found by Hosseini et al. (2013) when encapsulating Satureja hortensis essential oil were lower (from 52.4 to 66.37%) as they used essential oil concentrations below 5%, multiple emulsions, and emulsifying agents.

| Experimental runs | Concentration Cl2Ca (% p/p) | Time (min) | Efficiency (% ± S) | Yield (% ± S) |
|-------------------|-----------------------------|------------|--------------------|---------------|
| 1                 | 2 (-1)                      | 30 (-1)    | 95.63±2.63         | 59.06±1.93    |
| 2                 | 2 (-1)                      | 45 (0)     | 94.47±0.67         | 46.43±5.05    |
| 3                 | 2 (-1)                      | 60 (+1)    | 100.00±0.00        | 42.42±0.67    |
| 4                 | 5 (0)                       | 30 (-1)    | 95.44±1.66         | 68.20±3.42    |
| 5                 | 5 (0)                       | 45 (0)     | 98.59±0.11         | 68.18±0.79    |
| 6                 | 5 (0)                       | 60 (+1)    | 99.58±0.59         | 52.74±1.88    |
| 7                 | 10 (+1)                     | 30 (-1)    | 95.14±1.62         | 48.55±6.77    |
| 8                 | 10 (+1)                     | 45 (0)     | 95.29±1.09         | 53.04±6.31    |
| 9                 | 10 (+1)                     | 60 (+1)    | 100.00±0.00        | 59.61±2.29    |

Note. Efficiency and yield: mean% ± mean standard deviation, results of four measurements in each case.

Table 1: Levels of experimental design and results of encapsulation yield and efficiency

Optimization of the microencapsulation process

Efficiency and yield were maximized by the optimization of the process of obtaining grapefruit essential oil microcapsules. In the present study, it was applied the response surface methodology to a second-order 3^k factorial design with two replications. A total of 36 runs
were performed. The statistical analysis showed that the models for encapsulation efficiency and yield were appropriate (Table 2), with a significant R2 (0.85 and 0.83 respectively) and with a p-value of lack of fit larger than 0.05 (α-value) in both cases. The time variable had a significant linear effect (with a p-value smaller than 0.05) on both dependent variables (yield and efficiency). While the calcium chloride concentration did not have a significant linearly influence in any of them (p-value larger than 0.05), it influenced in a quadratic way on the encapsulation yield. Additionally, the quadratic effects were relevant to efficiency. Furthermore, the factors did not have significant interactions in the encapsulation efficiency, so this term could be removed from the mathematical model. However, it is possible that some kind of interaction between these factors existed in the case of the yield.

| Factor         | Encapsulation Efficiency | Encapsulation Yield |
|----------------|--------------------------|---------------------|
| Source         | Sum of squares | d.f. | Mean square | F value | P value | Sum of squares | d.f. | Mean square | F value | p value |
| Model          | 85.57         | 5    | 17.11       | 19.03   | 0.000024 | 1071.12       | 5    | 214.224   | 11.78   | 0.000272 |
| Linear         | 64.73         | 2    | 32.33       | 35.99   | 0.000008 | 135.59        | 2    | 67.795    | 3.73    | 0.055037 |
| A              | 0.888        | 1    | 0.89        | 0.99    | 0.339798 | 35.82         | 1    | 35.824    | 1.97    | 0.185775 |
| B              | 63.84        | 1    | 63.84       | 70.99   | 0.000002 | 99.77         | 1    | 99.765    | 5.49    | 0.037226 |
| Quadratic      | 19.97        | 2    | 9.98        | 11.1    | 0.001866 | 479.98        | 2    | 239.988   | 13.2    | 0.000932 |
| AA             | 4.81         | 1    | 4.82        | 5.35    | 0.039205 | 473.33        | 1    | 473.329   | 26.03   | 0.000261 |
| BB             | 15.15        | 1    | 15.15       | 16.85   | 0.001460 | 6.65          | 1    | 6.648     | 0.37    | 0.556673 |
| Interaction    | 0.13         | 1    | 0.13        | 0.14    | 0.713368 | 435.16        | 1    | 435.159   | 23.93   | 0.000371 |
| AB             | 0.13         | 1    | 0.13        | 0.14    | 0.713368 | 435.16        | 1    | 435.159   | 23.93   | 0.000371 |
| Residual       | 10.79        | 12   | 0.9         |         |           | 218.2         | 12   | 18.183    |         |          |
| Lack of fit    | 4.89         | 3    | 1.63        | 2.49    | 0.126413 | 105.51        | 3    | 35.171    | 2.81    | 0.100277 |
| Pure error     | 5.9          | 9    | 0.66        |         |           | 112.69        | 9    | 12.521    |         |          |
| Corrected total| 96.36       | 17   |             |         |           | 1289.32       | 17   |           |         |          |
| Correlation coefficient (R²) | 0.852     |     |             |         |           | 0.830         |     |           |         |          |
| Adjusted R²    | 0.79         |     |             |         |           | 0.76          |     |           |         |          |

Note. A: CaCl₂ concentration and B: crosslinking time (Independent variables). d.f.: Degree of freedom.

Table 2: Analysis of variance for encapsulation: efficiency and yield

The parameters with the significant coefficients of the efficiency (EE) and yield (R) encapsulation models obtained through the method of least squares are shown in the regression equations (7) and (8), both in uncoded units.

\[ EE\% = 103.88 + 0.95A - 0.62B - 0.07AA + 0.01BB \] (7)
\[ R\% = 65.60 + 3.75A - 0.41B - 0.07AA - 0.01BB + 0.12AB \] (8)

Based on these models, were created the response surface graphics (Figures 2 a-d) to understand the correlation between the results and the levels of each factor (calcium chloride concentration and crosslinking time). As shown in Figure 2a, higher efficiency values were obtained with times of 60 minutes and any value of calcium chloride concentration, whereas the higher yield zone had calcium chloride concentrations between 3 and 7.5% w/v (Figure 2b). The 3D response surface showed the surface curvature according to the second-order model. Figure 2c shows that at times of 60 minutes, there were obtained efficiencies between 99 and 100%, meaning that it was an optimal value of the crosslinking time. Figure 2d shows an optimal curvature point in less than 60 minutes.

The optimization of both variables through the desirability function is shown in Figure 3. The compound desirability (0.84) was good, which means that the combined values of the variables allowed efficiency and yield maximization. However, the individual desirability indicated that the configuration was more effective to maximize efficiency (d=1) than the yield (d=0.71). According to this model, with a calcium chloride concentration of 7.4% w/v and a crosslinking time of 58 minutes, it would be possible to reach a yield of 62% and efficiency of 100% of the encapsulation process. The prediction interval obtained for the yield was 57.62% to 72.86% and for the efficiency of 97.83% to 102.25%, with a confidence level of 95%.

![Fig. 2: The plot of (a) outline of Efficiency (E%), (b) outline of Yield (R%), (c) response surface for Efficiency (E%) and (d) response surface for Yield (R%) affected by calcium chloride concentration (A) and crosslinking time (B)](image-url)
Loss of water in the crosslinking process

The moisture analysis was performed in quintuplicate, with a weight around 0.8 g of microcapsules (without grapefruit oil, Cl2Ca solution of 7.4% w/v, crosslinking time of 58 minutes). The average percentage moisture of microcapsules was 79.5% ±1.7% and, compared to the 99% w/w initial amount of water in the emulsion, the average water loss was 19.4% w/w. These results might be one of the reasons behind the low yield in the obtention of microcapsules.

Morphology

The microcapsules obtained by the optimized process were observed using an optical microscope equipped with a digital camera. The diameters of 50 microcapsules were measured using a data acquisition system. The average value of the measured diameter was 1.95 mm ± 0.02 mm. Figure 4a shows the optical images of the microcapsule, its spherical shape and the swollen areas due to the essential oil.

Figures 4b–d represent SEM images with different magnifications showing the porous surface of the microcapsule and a considerable number of lumps due to the presence of oil. It can also be seen the porous surface like a sponge, indicating the sodium alginate crosslinking. Similar results were obtained by Soliman et al. (2013) in the encapsulation of clove and thyme essential oil.
Calcium adsorption in crosslinking

The crosslinking speed was studied in a calcium chloride solution of 7.5% p/v. The average values of adsorption of calcium ions in different crosslinking times are shown in Figure 5. The adsorption speed curve followed an exponential trend and reached equilibrium at 15 minutes of crosslinking. It was assessed the empirical data fitted to the Elovich model, generally used to model the kinetics of chemisorption of solutes in the liquid phase over solids. Then, an R2 of 0.997 was obtained, which indicated a very good fitting as it is shown in Figure 5. Then, the coefficients for the integrated form of the Elovich equation were calculated as follows (9):

![Micrographs of the optimized microcapsule](image)

**Fig. 4:** Micrographs of the optimized microcapsule (with 1% (w/v) sodium alginate, 10% (w/w) grapefruit oil, 7.5% (w/v) CaCl₂, and 58 min of crosslinking), (a) Optical image and, (b), (c) and (d) SEM (75x, 700x and 1000x magnification respectively)
\[ q_t = \frac{1}{4.66} \ln(22.77 \times 4.66 \times t) \] (9)

The value of the initial adsorption speed was 22.77 g·mg⁻¹·min⁻¹. The value of the adsorption constant (related to the square surface and the activation energy for chemisorption) was 4.66 g·mg⁻¹. While no studies were found that showed the kinetics of calcium adsorption in the crosslinking process, other works considered the adsorption speed of other metals over solid organic surfaces that fitted the Elovich model as well (Pinzón Bedoya and Vera Villamizar, 2009; Pérez-Marín et al., 2007). This model describes the chemisorption processes and supposes that the active sites of sodium alginate are heterogeneous with different activation energies (Ozacar and Şengil, 2005; Cheung et al., 2001). Figure 5 shows fast chemisorption at the beginning due to the formation of dimers that create the egg-box structure of the calcium alginate; but after five minutes, it slowed down considerably. This was explained by a progressive and uniform introduction of calcium ions caused by an increase of the surface crosslinking and continuing stirring (Teng and Hsieh, 1999). At the same time, the aggregation of the dimers led to the formation of an organized three-dimensional structure of bonds without heterogeneous grouping (Farrés and Norton, 2014).

This could also explain the influence of the crosslinking time in the efficiency of the encapsulation process. Longer crosslinking times would lead to a crosslinking network of greater order and degree of the three-dimensional “egg-box” packing, maximizing the oil transfer to the surface of the microcapsule.

**Controlled Release**

The controlled release study was performed with the microcapsules obtained at optimum conditions. The release of the essential oil microencapsulated in the alginate matrix was made on alcohol in order to ease its solubility. The samples taken at different times were analyzed by gas chromatography. Figure 6 shows the release compound percentage based

![Fig. 5: Fitting of experimental calcium adsorption data to the Elovich model](image-url)
on time for the microcapsules prepared. The release profile had two phases: a faster one at the start, or initial “burst”, and a lower one at the end (Siepmann et al., 2002). The first phase of the release could be attributed to a purely diffusional stage as a consequence of the volume expansion of the polymer due to the relaxation of its chains when submerged in liquids (Beirão-da-Costa, 2013; Hosseini et al., 2013). The almost zero-order release given below could be explained by a decrease in the release speed of the retained oil and the dispersion in the volume of the microcapsule (Hosseini et al., 2013) due to a greater distance to the surface of the microcapsule (Siepmann et al., 2002). Other authors obtained similar release profiles: with eucalyptus essential oil (Noppakundillograt et al., 2015) and with oregano essential oil (Ferrándiz García, 2015). The release rate grew at 83% in 270 minutes, being faster than the rate informed by Hosseini et al. (2013) from 60 to 78% in nine hours, with 1 to 3% v/v of the loading capacity of oil. This could stem from a greater oil loading capacity in the microcapsule, which would indicate a diffusion of larger quantities of oil molecules per pore (Banerjee et al., 2012). The total release of the microencapsulated oil (90 to 100%) was obtained between 200–360 hours of sustained release, but this was not illustrated in the graphics due to the scale used. This slower release by the end could be related to the size of the particle, as bigger particles had lower release rates due to the area-volume relation (Hosseini et al., 2013). The controlled release data obtained were fitted to the Korsmeyer and Peppas semi-empirical kinetic model (1981) and the kinetic parameters of the equation (10) were obtained through a statistical analysis using the method of least squares at a 95% confidence level:

\[
\frac{M_t}{M_\infty} = 2.08t^{0.475}
\]  

(10)

The value obtained of the coefficient of determination \(R^2\) was 0.991. The value of the diffusional exponent (n) showed an anomalous (no Fickian) transport release process from a sphere (Ritger and Peppas, 1987). This result suggested that the release process was controlled by the diffusion-relaxation processes of the polymer chains, according to the results from other authors (Noppakundillograt et al., 2015; Ráquira and Yadira, 2014; Escobar et al., 2002).

Fig. 6: Controlled release of grapefruit essential oil: at 25 °C from the microcapsules prepared with 1% (w/v) sodium alginate and 10% (w/w) grapefruit essential oil with 7.5% (w/v) CaCl2 for 58 min crosslinking time.
Conclusion

The microencapsulation of essential grapefruit oil with sodium alginate, by external ionic gelation and extrusion, was optimized with a loading capacity of oil of 10% w/w. The validation of the optimum process, tested with the desirability function, resulted in average efficiency of 99.97±0.02% and an average yield of 61.5±1.5%. In addition, it was possible to achieve an effective controlled release in time of the essential oil encapsulated. These results positively encourage the possible use of microencapsulated grapefruit essential oil in the food industry for the conservation of certain types of dry foods.

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