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## Characterization of Core-Shell Alginate Capsules

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# Food Biophysics

## CHARACTERIZATION OF CORE-SHELL ALGINATE CAPSULES GENERATED USING DROPLETS MILLIFLUIDIC

--Manuscript Draft--

<b>Manuscript Number:</b>	FOBI-D-19-00051R1	
<b>Full Title:</b>	CHARACTERIZATION OF CORE-SHELL ALGINATE CAPSULES GENERATED USING DROPLETS MILLIFLUIDIC	
<b>Article Type:</b>	Original Research	
<b>Keywords:</b>	Millifluidic; Encapsulation; inverse gelation; alginate; sunflower oil	
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<b>Funding Information:</b>	Consejo Nacional de Innovación, Ciencia y Tecnología (not available)	Dr Mariana Pereda
<b>Abstract:</b>	<p>A new droplets millifluidic/inverse gelation based process was used to produce core-shell alginate milli-capsules. Water-in-oil (W/O) emulsion dispersed phase containing Ca<sup>2+</sup> ions was directly injected into a continuous alginate phase to generate a secondary W/O/W emulsion. Due to the cross-linking of alginate molecules by Ca<sup>2+</sup> ions release, core-shell milli-capsules were formed with a very high oil loading. The influence of the curing time and of the storage conditions on capsules physico-chemical properties was investigated.</p> <p>It was first found as expected that alginate membrane thickness increased with curing time in the collecting bath. However, a plateau was reached for the higher curing times, in close relation with previous observations (Martins, Poncelet, Marquis, Davy, &amp; Renard, 2017b) that an external oil layer surrounded the surface of W/O emulsion drops that acted as a barrier and hindered the release of aqueous CaCl<sub>2</sub> droplets during curing time. Compression experiments on individual capsules revealed that alginate membrane thickness was inversely related to its mechanical properties, i.e. the thicker membrane, the lower surface Young modulus. Surface Young modulus ranged from 61 to 26 N/m at curing times of 3 and 45 min, respectively. This result was explained in terms of enhanced swelling properties of alginate membrane with curing time or storage conditions. Drying capsules led to much more resistant membranes due to the loss of water. Oil loading of 80 wt% was obtained for dry capsules whatever the conditions used.</p>	

## Response to Reviewer 3 Comments

Point-by-point answers to reviewer 3 are given below and corrections appear in red in the revised manuscript.

Reviewer #3: I have reviewed this manuscript titled „CHARACTERIZATION OF CORE-SHELL ALGINATE CAPSULES GENERATED USING DROPLETS MILLIFLUIDIC" based on the innovativeness, quality, significance, and presentation of results. In general, the presentation of results is lacking. This paper could be published but only after the corrections and explanations.

Line 29. Change „was" to „were".

Changing was made in the revised manuscript.

Line 63. Change „consist" to consists".

« Consists » appears in the original manuscript.

Line 85. Change „the microcapsules production" to „the production of the microcapsules".

Changing was made in the revised manuscript.

Line 126. Change „were" to „was".

Changing was made in the revised manuscript.

Line 150. Were capsules washed before storage in Ca<sup>2+</sup> or Na<sup>+</sup> containing solutions?

According to reviewer's remark, the following sentence was changed in the revised manuscript "the capsules were sieved, rinsed with distilled water and suspended in a Ca<sup>2+</sup> or Na<sup>+</sup> solution".

Line 165. Change „microscopy" to „microscope".

Changing was made in the revised manuscript.

Line 224. Change „hydrophilic lipophilic" to „hydrophilic-lipophilic".

Changing was made in the revised manuscript.

Line 236. High-pressure homogenization? Where is this included in the paper? There is no mentioning of High-pressure homogenization throughout the paper.

We agree with reviewer 3 and the following changing was made in the revised manuscript « High shear mixing... »

Figure 5 and Figure 7. Are there statistically significant differences with regards to the curing time and membrane thickness/capsule diameter? Are there statistically significant differences with gelation time? These should be added to the figures and comment in the discussion.

Authors performed statistical analyses on data displayed in Figures 5 and 7. The following sentence was therefore added in the Figure captions of the revised manuscript : «At the 0.05 level, the populations means were significantly different ». In addition, authors added in the caption of Figure 5 « Solid line corresponded to the fitting of the data by a linear function; dotted line was just eye-guide. » Authors also replaced « Gelation time » by « Curing time » in Figure 7. Finally, additional comments regarding statistical analyses were added in the revised manuscript.

Table 2 and Figure 9 seem to have a repetition of the data for membrane thickness. Also are there significant differences found between 3h, 24h, and 48h in Figure 9?

The repetition of the data between Table 2 and Figure 9 is only true for data obtained after 48h storage of capsules. Figure 9 therefore also displayed evolution of membrane thickness in time after 3, 24 and 48h storage of capsules.

Authors performed statistical analyses on data displayed in Figure 9. The following sentence was therefore added in the Figure caption of the revised manuscript : «At the 0.05 level, the populations means were significantly different ».

Regarding the writing, some corrections should be applied to add/remove articles the/a/an.



Changes were made according to reviewer 3 where articles « the/a/an » were in strikethrough text in the revised manuscript (or sometimes added in the revised manuscript).

## Response to Reviewer 4 Comments

Point-by-point answers to reviewer 4 are given below and corrections appear in red in the revised manuscript.

### General comments

The objects of the paper are interesting and it makes sense to investigate the droplet characteristics of capsules formed by inverse gelation in more detail. However as Ca-alginate gels are highly porous gels, I seriously doubt about the protective effect of a Ca-Alginate layer against oxygen and water. What is the mean pore size of this protective layer? How can the gel protect against the diffusion of water?

Authors have no doubt about the porosity of alginate beads for which values ranging from 5 to 200 nm were obtained using TEM (Andresen et al. ACS Symp. Ser. 1977; 48 361-381) while values ranging from 12 to 16 nm were obtained using size exclusion chromatography (Klein et al. Eur. J. Appl. Microbiol. Biotechnol. 1983 18 86-91). The differences in porosity were explained by a difference in porosity between outside (pores of small size) and inside (pores of large size) of alginate beads (Smidsrod, Carb. Res. 1973 27 107-118). Authors suppose that the differences in porosity identified by Smidsrod could also exist in alginate membrane of capsules. The wide distribution of pores size therefore gives alginate beads and capsules a wide range of applications and in particular in drug delivery applications.

### Specific comments

p. 4 line 76: the solution could also be sprayed instead of dripped to get smaller capsules

Authors totally agree with reviewer 4. The following sentence was therefore added in the revised manuscript: "Alternative to reduce size is therefore to spray alginate solution rather than dripping to a bath of CaCl<sub>2</sub> solution [7]."

The objective of the work is not made clear.

According to reviewer 4, authors have modified lines 105 to 109 to make objective of the work clearer.

The objective of the present work is, therefore, to produce core-shell alginate milli-capsules using a new droplets millifluidic/inverse gelation based process. The contribution tends to analyze the effect of varying curing time and cation concentration of the storage solution on the final properties of the obtained capsules, including oil content and mechanical properties.

p. 5 line 107 To study the effect of cation concentration is not mentioned at all in the introduction part and it seems that this study was added to explain the change in young modulus with shell thickness

The effect of cation concentration is mentioned page 5 line 107 "...varying the curing time and the cation concentration of the storage solution...".

2.1 line 117 ff: why is tween 20 not mentioned?

Tween 20 (Sigma Aldrich, France) was therefore added in the material section line 122 in the revised manuscript.

2.3.3 line 179: Is alginate solution Newtonian?

Authors have determined that low concentration solution of alginate at C = 1% and sunflower oil were both Newtonian while W/O emulsion displayed a shear-thinning behaviour (data not shown as authors thought that it did not bring useful information to understand the contribution).

Table 1: What is D



D corresponds to the capsule diameter. Table 1 was therefore corrected as follows in the revised manuscript:

	<b>Diameter D (mm)</b>	<b>Membrane thickness m (<math>\mu</math>m)</b>	<b>D/m</b>	<b>Surface Young modulus (N/m)</b>	<b>Force at break (N)</b>
Wet capsules	2.21 $\pm$ 0.07	297 $\pm$ 76	7.4 $\pm$ 0.3	26.1 $\pm$ 1.8	1.15 $\pm$ 0.27
Dry capsules	1.85 $\pm$ 0.14	27 $\pm$ 12*	60.1 $\pm$ 5.9	613 $\pm$ 163	1.45 $\pm$ 0.37

Fig. 3 curves are difficult to distinguish

Authors agree with reviewer 4 and Figure 3 was redrawn in the revised manuscript by plotting data with different colours.  
p. 12 285: How was the oil content of the dried capsules quantified?

Oil content was determined by TGA for both wet and dried capsules. The sentence in the revised manuscript was rephrased as follows: "By this way, it was therefore possible to use this technique to determine the oil content of the wet and dried capsules between 300 and 510°C".

Fig.4: Why did you choose different regions of interest and different magnifications?

Micrographs were taken at different regions of interest in order to highlight membrane growth and heterogeneity as function of curing time. Scale bar was constant at 250  $\mu$ m for all micrographs.

Fig. 5: How is polydispersity defined and where do I find this value in the plot? I don't see an exponential increase as indicated in the text but a linear increase and saturation above a certain curing time

Authors totally agree with remark of reviewer 4 as polydispersity was not properly defined in the manuscript. Size polydispersity resulted from size measurements of capsule diameters using microscopy and could be ascribed to the standard deviation divided by the average size. The following sentence was therefore added in the revised manuscript: "The average diameter of alginate capsules ranged from 1.9 to 2.6 mm depending on curing time (significant differences at  $p < 0.05$ ), with a size polydispersity lower than 10% (i.e. standard deviation / average size)".

Regarding the evolution of membrane thickness versus curing time, authors totally agree with the remark of reviewer 4. Authors therefore rephrased the sentence s follows in the revised manuscript: "From Figures 4 and 5, it was concluded that capsule diameter and membrane thickness were linearly dependent on curing time; in addition, membrane thickness reached a plateau at high curing times due to restricted calcium diffusion."

Table 2: Units are missing

Authors corrected Table 2 in the revised manuscript as follows:

<b>Storage solution (wt%)</b>	<b>Diameter (d, mm)</b>	<b>Membrane thickness (Mt, <math>\mu</math>m)</b>	<b>d/Mt</b>	<b>Surface Young modulus (Es, N/m)</b>	<b>Es/Mt (N/m/<math>\mu</math>m)</b>
Distilled Water	2.27 $\pm$ 0.20 <sup>a,c</sup>	479 $\pm$ 74 <sup>a</sup>	4.7 $\pm$ 0.3 <sup>a,d</sup>	23.4 $\pm$ 2.64 <sup>a</sup>	0.049
CaCl <sub>2</sub> 0.1	2.43 $\pm$ 0.17 <sup>a,b,c</sup>	388 $\pm$ 84 <sup>a,b,c</sup>	6.3 $\pm$ 1.7 <sup>a,b,c</sup>	28.7 $\pm$ 4.6 <sup>a</sup>	0.073
CaCl <sub>2</sub> 0.3	2.33 $\pm$ 0.18 <sup>a,b,c</sup>	316 $\pm$ 44 <sup>b,c</sup>	7.4 $\pm$ 1.4 <sup>b,c</sup>	39.5 $\pm$ 3.3 <sup>b,c</sup>	0.125
CaCl <sub>2</sub> 1	2.40 $\pm$ 0.19 <sup>a,b,c</sup>	392 $\pm$ 61 <sup>a,b,c</sup>	6.1 $\pm$ 1.1 <sup>a,b,c</sup>	29.1 $\pm$ 6.6 <sup>a,b</sup>	0.074
CaCl <sub>2</sub> 1.5	2.60 $\pm$ 0.14 <sup>b</sup>	345 $\pm$ 48 <sup>c</sup>	7.5 $\pm$ 1.1 <sup>c</sup>	42.6 $\pm$ 6.9 <sup>c</sup>	0.123
NaCl 1.5	2.11 $\pm$ 0.14 <sup>c</sup>	748 $\pm$ 96 <sup>d</sup>	2.8 $\pm$ 0.5 <sup>d</sup>	2.3 $\pm$ 0.1 <sup>d</sup>	0.003

Fig. 8a: I can't see a linear decrease and the linear regression line is not justified at all!

Authors agree with remark of reviewer 4 where linear fitting of data in Figure 8a was highly speculative. The following figure was included in the revised manuscript where a discontinuous line was added as an eye-guide:



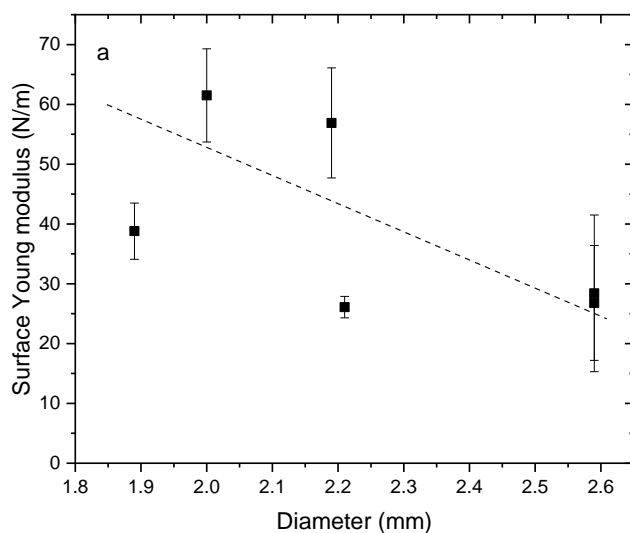


Fig. 8b and 8c: No plateau can be found as indicated in the text

Authors agree with remark of reviewer 4. This mistake was therefore corrected in the revised manuscript as follows: “This phenomenon was clearly seen on Figures 8b and 8c where a linear decrease was noticed for the surface Young modulus with the increase of membrane thickness and diameter / thickness ratio.”

p. 18 line 419: How does the young modulus change with curing time for dried microcapsules?

Measurements of the Young modulus were performed on dried capsule only at constant curing time (30 min).



1 **CHARACTERIZATION OF CORE-SHELL ALGINATE CAPSULES GENERATED**  
2 **USING DROPLETS MILLIFLUIDIC**

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7 4 Mariana Pereda <sup>(1,2,3)</sup>, Denis Poncelet <sup>(2)</sup>, Denis Renard\* <sup>(3)</sup>

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23 **ABSTRACT**

1  
2 24 A new droplets millifluidic/inverse gelation based process was used to produce core-shell  
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4 25 alginate milli-capsules. Water-in-oil (W/O) emulsion dispersed phase containing  $\text{Ca}^{2+}$  ions  
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7 26 was directly injected into a continuous alginate phase to generate a secondary W/O/W  
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10 27 emulsion. Due to the cross-linking of alginate molecules by  $\text{Ca}^{2+}$  ions release, core-shell milli-  
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12 28 capsules were formed with a very high oil loading. The influence of the curing time and of the  
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14 29 storage conditions on capsules physico-chemical properties were investigated.

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17 30 It was first found as expected that alginate membrane thickness increased with curing time in  
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19 31 the collecting bath. However, a plateau was reached for the higher curing times, in close  
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21 32 relation with previous observations (Martins, Poncelet, Marquis, Davy, & Renard, 2017b) that  
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23 33 an external oil layer surrounded the surface of W/O emulsion drops that acted as a barrier and  
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25 34 hindered the release of aqueous  $\text{CaCl}_2$  droplets during curing time. Compression experiments  
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27 35 on individual capsules revealed that alginate membrane thickness was inversely related to its  
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29 36 mechanical properties, i.e. the thicker membrane, the lower surface Young modulus. Surface  
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31 37 Young modulus ranged from 61 to 26 N/m at curing times of 3 and 45 min, respectively. This  
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33 38 result was explained in terms of enhanced swelling properties of alginate membrane with  
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35 39 curing time or storage conditions. Drying capsules led to much more resistant membranes due  
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37 40 to the loss of water. Oil loading of 80 wt% was obtained for dry capsules whatever the  
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39 41 conditions used.

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48 43 **KEYWORDS:** Millifluidic; encapsulation; inverse gelation; alginate; sunflower oil.  
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48 **1. INTRODUCTION**

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2 49 The study of microcapsules based on biodegradable polymers is of great importance in  
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5 50 current scientific research worldwide due to the unusual combination of properties and  
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7 51 versatility of these materials.

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9 52 Microencapsulation avoids the degradation and volatilization of bioactives, protecting  
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11 53 and preserving their biological activity and active functional ingredients. The  
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14 54 microencapsulation technique has been mainly described as a process in which small particles  
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17 55 or droplets are surrounded by a homogeneous or heterogeneous coating, forming beads or  
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19 56 capsules with various applications [1]. There are three major fields of application: food  
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22 57 production, e.g. the enhancement of nutrition by encapsulated vitamins, minerals or even pro-  
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24 58 biotic bacteria; the cosmetic industry, where encapsulation of colors and flavors became more  
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27 59 and more popular within the last years; and the drug delivery field.

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29 60 Oils are widely applied in the formulation of foods, pharmaceutical and cosmetic  
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32 61 products; however, they are often volatile, labile and sensitive to environmental factors such  
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34 62 as heat, light, water and oxygen [2]. An efficient strategy to decrease their sensitivity towards  
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37 63 environmental conditions consists of its encapsulation in inert polymer matrix using  
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39 64 gelation/emulsification technique [3-5].

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41 65 Alginate is one of the most widely used biocompatible polymers in  
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44 66 microencapsulation, as it forms a highly versatile, biocompatible and nontoxic matrix of gel  
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46 67 that protects the active components of factors such as heat and moisture thereby enhancing  
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49 68 stability and bioavailability. Alginates are algal polysaccharides consisting of a linear chain of  
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51 69 (1-4) linked residues of  $\beta$ -D-mannuronic acid (M) and  $\alpha$ -L-guluronic acid (G) in different  
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54 70 proportions and sequential arrangements [6]. This polymer has a remarkable property of quick  
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56 71 gelation in presence of divalent ions such as calcium ions ( $\text{Ca}^{2+}$ ).

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72 Among the different methods used to prepare beads of gelled alginate, the most widely  
73 used encapsulation method is the simple extrusion/external gelation [7-8], wherein the  
74 formation of beads containing the solution of alginate and the active to be encapsulated is  
75 accomplished by using an extruder device dripping to a bath of CaCl<sub>2</sub> solution which induces  
76 alginate gelation [1]. The main limitations of this technique are the large size of beads formed,  
77 which depends on the diameter of the nozzle extruder, the large quantity of oil used to  
78 generate emulsion drops which is generally wasted, and the difficulty of large scale  
79 production because beads are formed one by one. **One alternative to reduce size is therefore to  
80 spray alginate solution rather than dripping to a bath of CaCl<sub>2</sub> solution [7].**

81 On the other hand, in the inverse gelation mechanism, oil and CaCl<sub>2</sub> solution are  
82 emulsified and added dropwise into an alginate solution bath [9], where Ca<sup>2+</sup> ions diffuse  
83 from the emulsion drop to the alginate bath cross-linking the surrounding alginate molecules  
84 [9]. This technique allows producing core-shell capsules with an oil loading of nearly 100%  
85 (v/v) for dry capsules by using the dispersion-inversion gelation technique [10]. In addition,  
86 the **production of microcapsules** using droplets millifluidic is a promising strategy for oil  
87 encapsulation and allows the easy production of highly controlled and uniform microcapsules  
88 from W/O emulsions [11]. A similar droplets millifluidic set-up was used to produce core-  
89 shell alginate capsules with an aqueous core instead of an oil core [12]. The millifluidic  
90 device used in [11] consisted in a co-axial flow focusing geometry; water-in-oil (W/O)  
91 emulsion drops containing Ca<sup>2+</sup> are directly injected through a capillary or needle into the co-  
92 flowing continuous phase (alginate solution) to form ~~the~~ micro-capsules downstream of the  
93 circuit. The oil encapsulation using this gelation mechanism is a very recent approach and is  
94 still scarcely studied [9, 2, 11]. For example, Martins et al. (2017b) studied the different  
95 factors affecting ~~the~~ emulsion stability, ~~the~~ capsule size and ~~the~~ membrane thickness. This  
96 study demonstrated the success of the production of (micro)-capsules based on the inverse

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97 gelation mechanism using droplets millifluidic. Monodisperse (micro)-capsules with sizes  
98 ranging from 1.4 mm down to 140  $\mu\text{m}$  were produced. The present work can be considered as  
99 a continuation of the work of Martins et al (2017b), where the focus was on the final  
100 properties of capsules and more specifically on the mechanical properties and oil content of  
101 the capsules. In many cases, the capsules are subjected to mechanical stresses exerted by their  
102 environment that induce deformation and potential breakup. It is important to control the  
103 mechanical properties of capsules to ensure protection and/or release of the encapsulated  
104 substances under proper conditions and also to avoid breakup during processing. The present  
105 study focused on capsules made of a deformable membrane with an internal liquid core.

106         The objective of the present work is, therefore, to take advantages of the new droplets  
107 millifluidic/inverse gelation based process to produce core-shell alginate milli-capsules and to  
108 analyze the effect of varying the curing time and the cation concentration of the storage  
109 solution on the final properties of the obtained capsules, including oil content and mechanical  
110 properties.

111         The use of millifluidic devices paves the way to an integrative formulation of core-  
112 shell materials with a broad size range of monodisperse capsules (from  $\mu\text{m}$  to mm) and new  
113 complex architectures. This should lead to the rapid emergence of new products in cosmetics  
114 or food, where the texture and visual aspect play a key role for sale.

## 115 116 **2. MATERIALS AND METHODS**

### 117 ***2.1. Materials:***

118 Sodium alginate powder (Saltialgine S 60 NS) with a mannuronic (M) to guluronic (G) acid  
119 unit ratio (M/G) and a molar mass equal to 1.37 and  $1.57 \times 10^5$  g/mol, respectively, was  
120 kindly donated by Cargill (France). Calcium chloride powder ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) (Panreac  
121 Quimica Sau, Spain), sunflower cooking oil (Associated Oil Packers, France), PGPR 90

122 (Danisco, France), Tween 20 (Sigma Aldrich, France) were used to prepare the W/O  
123 emulsions. Other chemicals reagents were obtained from Sigma Aldrich (France).

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## 125 **2.2. Methods:**

### 126 *2.2.1. Preparation of alginate and calcium chloride solutions*

127 Ten grams of alginate powder was dissolved in 1 L of demineralized water using a magnetic  
128 stirrer. Tween 20 (0.5%, v/v) was added to the alginate solution. The calcium chloride  
129 solution was prepared by dissolving 240 g of CaCl<sub>2</sub>·2H<sub>2</sub>O in 1 L of demineralized water.

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### 131 *2.2.2. Preparation of the W/O emulsions*

132 Emulsions were prepared according to Martins et al. (2017b). One hundred millilitres of  
133 sunflower oil containing 0.96 g of surfactant (PGPR 90) was mixed using a high shear mixer  
134 (Ultra-Turrax T25, IKA, Germany) at 18 000 rpm for 1 min. Sixty millilitres of calcium  
135 chloride solution (240 g/L) was then added slowly and a new shear mixing at 18 000 rpm for  
136 3 min was performed.

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### 138 *2.2.3. Capsules production by droplets millifluidic*

139 A millifluidic device with a co-axial flow focusing geometry was used as described in [11]  
140 (Figure 1, Supplementary data). The dispersed phase, a W/O emulsion freshly prepared (see  
141 section 2.2.2.) was pumped (Harvard Apparatus PHD 2000, France) through a fused silica  
142 capillary tube (interior diameter (ID) 530 μm and outside diameter (OD) 660 μm) at a rate  
143 ( $Q_{emul}$ ) of 4 mL/h. The continuous phase, an alginate solution added with Tween 20, was  
144 pumped through a Teflon tube (ID=1.57 mm and OD= 0.5 mm) at a rate ( $Q_{alg}$ ) of 10 mL/h.  
145 The W/O emulsion and the alginate solutions co-flowed in the glass tube (ID= 2 mm, OD= 4  
146 mm and length of 10 cm). As the generation of capsules using W/O emulsion requires

147 hydrophilic surfaces, the glass tube was previously immersed in a saturated-NaOH solution  
148 for 5 min at room temperature and rinsed using tap water [13].

149 W/O emulsion drops were formed and dispersed in the continuous alginate phase. Calcium  
150 ions ( $\text{Ca}^{2+}$ ) diffused from the W/O emulsion drop cross-linking the alginate molecules. The  
151 capsules were collected in an alginate bath stirred at 50 rpm. After a curing time (time of  
152 contact between the capsules and the alginate solution) varying between 3 and 50 min, the  
153 capsules were sieved, **rinsed with distilled water**, suspended in a  $\text{Ca}^{2+}$  or  $\text{Na}^+$  solution or in  
154 distilled water and stored at  $4^\circ\text{C}$  until use.

155

### 156 ***2.3. Characterization of the W/O emulsions***

#### 157 *2.3.1. Stability*

158 ~~the~~ W/O emulsion stabilities were performed as described by Martins et al. (2015). Hundred  
159 millilitres of W/O emulsions were placed in tubes of 100mL with graduation of 1mL. The  
160 emulsions were kept at ambient temperature ( $20 \pm 2^\circ\text{C}$ ) and visually inspected as a function of  
161 time in order to assess the critical time for which 1% of phase separation occurred (i.e.  
162 corresponding to 1 mL of phase separated liquid).

163

#### 164 *2.3.2. Microscopic observations*

165 W/O emulsions were diluted ten times in sunflower oil and observed under the microscope.  
166 The diluted emulsions were brought on a **microscope** slide using a capillary after which a  
167 cover slip was gently applied. Image acquisition was conducted using an optical microscope  
168 (Leica Microsystems, France). The size of  $\text{CaCl}_2$  solution droplets (dispersed phase) was  
169 determined by image analysis using the ImageJ 1.47v freeware (National Institutes of Health,  
170 Bethesda, Maryland, USA). ~~the~~ **M**Measurements were carried out in triplicate with three  
171 repetitions. In other words, three W/O emulsions of each formulation were analyzed by

172 optical microscopy. Three different zones ( $1.1 \times 1.5$  mm) for each sample were imaged and  
173 approximately one hundred droplets per zone were measured.

174

### 175 2.3.3. Rheological measurements

176 The viscosity of sunflower oil, alginate solution and water in-oil emulsions were investigated  
177 with a stress-controlled rheometer (AR-2000, TA Instruments, New Castle, DE) equipped  
178 with a Couette geometry (DIN 412). The temperature was controlled with a Peltier system.  
179 ~~the~~ Dynamic viscosity and shear stress as a function of shear rate between  $0.01$  and  $1000 \text{ s}^{-1}$   
180 were measured. Viscosity of alginate solution at  $C = 1\%$  (w/v) gave  $\eta = 35.9 \pm 3.9 \text{ mPa}\cdot\text{s}$   
181 while that of sunflower oil was of  $70.2 \pm 1.7 \text{ mPa}\cdot\text{s}$ . W/O emulsion had a viscosity  $\eta = 212.5$   
182  $\pm 7.4 \text{ mPa}\cdot\text{s}$ .

183

## 184 ***2.4. Characterization of capsules***

### 185 *2.4.1. Microscopic observation*

186 The External diameter (D), and membrane thickness (m) of capsules were performed under an  
187 optical microscope (Olympus IX51, France) equipped with a camera (Olympus DP70). A 40X  
188 objective was used to image microcapsules.

189 Dp controller software (version 2.1.1) was used for images acquisition. Shell thickness ( $M_t$ )  
190 and capsules diameter (d) were therefore calculated from image analyses on 10 capsules per  
191 experimental condition using the imageJ 1.47v freeware (National Institutes of Health,  
192 Bethesda, Maryland, USA).

193

### 194 *2.4.2. Total oil content of the capsules*

195 A TGA Q5000 instrument was used for the thermo-gravimetric analysis (TGA). An  
196 isothermal step at  $60^\circ \text{ C}$  during 30 minutes was applied before the ramp up to  $800^\circ \text{ C}$  at

197 10 °C/min under a nitrogen atmosphere (25 mL/min). The capsules were dried 48 h at room  
198 temperature and  $a_w=0.59$  (NaBr) before TGA analyses. Oil content was determined by  
199 analyzing the thermograms of pure components and capsules.

#### 201 *2.4.3. Mechanical study*

202 A Dynamic Mechanical Analyzer (Rheometric Scientific, EEUU) equipped with a plate-plate  
203 geometry (diameter of 20 mm) was used. The upper plate had a diameter of 20 mm and the  
204 normal force was measured as a function of the compression in a range between 0.001 and 20  
205 N. A force gap test was used to compress the capsules with a linear compression speed of 0.6  
206 mm / min. The gap and the normal force being imposed were measured simultaneously at the  
207 upper plate. The surface Young modulus was calculated by a quantitative analysis of force–  
208 displacement curves at small deformations [14-15]. Five replicates were considered for each  
209 capsule.

#### 211 *2.4.4 Statistical analysis*

212 Data values obtained in the experiments were statistically analyzed by one-way analysis of  
213 variance (ANOVA) employing OriginLab 8.5 software. Differences between pairs of means  
214 were assessed on the basis of confidence intervals using the Tukey test. The least significance  
215 difference was  $P < 0.05$ .

### 218 **3. RESULTS AND DISCUSSION**

#### 219 *3.1. Characterization of the W/O emulsions*

220 Emulsions containing sunflower oil,  $\text{CaCl}_2$  solution (240 g/L) and PGPR 90 were prepared  
221 using a high shear mixer. The dispersed W/O phase contained the calcium content that allows

222 to crosslink the alginate and form the membrane of the capsules. ~~the~~ Knowledge of the  
1 stability is very important as it is directly related with the Ca<sup>2+</sup> release, and therefore the  
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5 224 membrane formation.

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7 225 PGPR 90 was chosen as emulsifier due to its low hydrophilic-lipophilic balance value  
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10 226 (HLB=1.5), which made it prone to stabilize W/O emulsions [16].

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12 227 Several images of the emulsion in function of storage time, formed by dispersing calcium  
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14 228 aqueous phase into the sunflower oil containing the surfactant PGPR 90, were shown in  
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16  
17 229 Fig. 1. Minimal phase separation and droplet coalescence occurred during storage over 24 h.  
18  
19 230 Martins et al (2017b) studied the effect of surfactant concentration on the stability of the  
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21  
22 231 emulsions. By increasing emulsifier concentration, W/O emulsions with stabilities increasing  
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24 232 from 0.9 to 132 h were found. Marquez et al. [17] and Su et al. [18] found that higher the  
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27 233 emulsifier concentration was, lower was the size of water droplets in the W/O emulsion,  
28  
29 234 increasing both its viscosity and stability.

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31 235 In addition to visual inspections of the emulsion stability, the drops size of the dispersed  
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34 236 aqueous phase was measured after 10 times dilution with oil by optical microscopy. Figure 2a  
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36 237 showed an image of a W/O emulsion stabilized by PGPR 90 at 6 g/L. High shear mixing led  
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39 238 to emulsions with a narrow size distribution and a mean diameter of the dispersed aqueous  
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41 239 phase drops of about 1.4 μm.

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46 241 In order to confirm that it is a W/O emulsion, a drop of the emulsion was dispersed in water  
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49 242 (Figure 2b) and analyzed under optical microscopy. A double W/O/W emulsion was formed  
50  
51 243 by an oil continuous phase and a first dispersed CaCl<sub>2</sub> solution droplets internal phase  
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53 244 surrounded by a second dispersed CaCl<sub>2</sub> external phase.

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58 246 **3.2. Characterization of components by TGA**



247 the Thermal degradation behavior of pure components that form the capsules (alginate,  
248 calcium and sunflower oil) was performed and analyzed by TGA up to 800°C, with the idea  
249 of identifying the degradation temperature of each component. Figure 3 showed the TGA  
250 thermograms and derivative thermograms dTGA of sunflower oil, CaCl<sub>2</sub> and alginate  
251 powders.

252 By analyzing the TGA of CaCl<sub>2</sub>·2H<sub>2</sub>O, a considerable weight loss was clearly observed  
253 between 25°C and 130°C that was ascribed to the endothermic removal of crystalline and  
254 loosely bound surface water, as it was also observed by Karunadasa et al. [19]. There was a  
255 first endothermic dehydration at around 130 °C accounted for the emergence of monohydrate  
256 calcium chloride (CaCl<sub>2</sub>·H<sub>2</sub>O) (Figure 3). A significant reduction in weight of calcium  
257 chloride was observed during the second endothermic dehydration (between 150 and 220 °C,  
258 dTGA curve, Fig. 3) that further ensured the complete removal of crystalline water from the  
259 material. Although the magnitude of second endothermic dehydration was significantly larger  
260 than the first one, it was concluded that the removal of crystalline water from the  
261 monohydrate was rather difficult compare to the dihydrate calcium chloride (Fig. 3, dTGA  
262 curve). This was a good indication of the strong association of crystalline water in  
263 monohydrate that requires a considerable amount of thermal energy to eliminate it from the  
264 monohydrate [19].

265 The TGA/dTGA curves for sunflower oil displayed two thermal decomposition steps in the  
266 range of 300 to 510°C, with no residue remaining at 800°C (see Figure 3). It was observed  
267 that the first step (300 to 420°C) corresponded to the decomposition of the polyunsaturated  
268 fatty acids. During heating, the triglycerides, which form 96 to 98% of the edible oils, produce  
269 volatile compounds, which are constantly removed by vapor generated during heating. These  
270 products (dimers, trimers, polymers) are formed principally by thermal reactions of  
271 unsaturated fatty acids, such as linoleic acid. The first step is the most important for the

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272 thermal stability of edible oils, because this is the step where decomposition of the  
273 unsaturated fatty acids begins. The thermal stability of sunflower oils is dependent on the  
274 composition of the fatty acids, as it is influenced by artificial antioxidants [20]. The beginning  
275 of oxidation in edible vegetable oils is characterized by absorption of oxygen through the fatty  
276 acid chain, subsequently forming the oxidation product as peroxides. This behavior is  
277 generally identified by an increase in the initial mass of the sample. The second step in the  
278 thermal decomposition of sunflower oils (420 to 520°C) corresponds to the decomposition of  
279 monounsaturated fatty acids such as oleic acid and saturated fatty acids, such as palmitic acid.  
280 During this reaction, the double bonds are broken, causing the triglyceride molecules in the  
281 edible vegetable oils to become saturated [20].

282 Figure 3 also showed the TGA and dTGA curves for the sodium alginate. It is shown that the  
283 salt decomposed by dehydration followed by degradation to  $\text{Na}_2\text{CO}_3$  and a carbonized  
284 material that decomposed slowly from 550-750 °C in  $\text{N}_2$  (Figure 3), as also noticed by other  
285 authors [21-22].

286 Taking into account that the alginate content in the dried capsules as well as the degradation  
287 peaks observed by dTGA curves are negligible, compared to the sunflower oil alone, it was  
288 considered that temperature range of the oil degradation did not overlap with those of alginate  
289 and  $\text{CaCl}_2$  pure components. By this way, it was therefore possible to use this technique to  
290 determine the oil content of the wet and dried capsules between 300 and 510°C.

### 291 **3.3. Microcapsules formation**

292 Based on Martins et al (2017b) previous results, the mechanism of capsule formation would  
293 occur in three steps:

294 I- the Emulsion drops characterized by a continuous oil phase containing  $\text{CaCl}_2$  solution  
295 droplets contacted the alginate solution

296 II- ~~the~~ CaCl<sub>2</sub> solution droplets near the oil-alginate solution interface were able to migrate  
297 from the emulsion to the alginate phase by a mechanism of diffusion/permeation through the  
298 oil layer [23]

299 III- ~~the~~ Ca<sup>2+</sup> ions cross-linked the alginate chains resulting in the formation of a membrane.

### 300 **3.3.1. Effect of curing time**

301 W/O emulsions containing 90 g/L of CaCl<sub>2</sub> and 6g/L of PGPR 90 were co-flowed with the  
302 alginate-Tween 20 continuous phase in order to generate monodisperse W/O emulsion drops  
303 that finally reached the alginate bath. These capsules were kept in contact with the alginate  
304 bath by increasing curing time from 3 to 50 minutes. After these curing times, capsules were  
305 filtered, rinsed with distilled water and stored in a CaCl<sub>2</sub> solution (15 g/L) at 4°C before ~~the~~  
306 microscopy observations.

#### 307 **3.3.1.1. Size of capsules and membrane thickness**

308 Microscopic observations revealed that spherical capsules with a well-defined alginate  
309 membrane were collected in the alginate bath whatever the curing time (Figure 4).

310 Figure 5 showed the dependence of ~~the~~ capsule diameter and ~~the~~ membrane thickness with ~~the~~  
311 curing time. The average diameter of alginate capsules ranged from 1.9 to 2.6 mm depending  
312 on curing time (**significant differences at  $p < 0.05$** ), with a size polydispersity lower than 10%  
313 (**data not shown**). Other authors also found that microcapsules produced using microfluidics  
314 had extremely narrow size distributions [24]. Using droplets millifluidic process, capsule size  
315 depends on droplet dimension before getting into contact with the alginate phase, ~~the~~ droplet  
316 dimension depending on both the internal diameter of the Teflon tube (which was constant  
317 and equal to 1.57 mm throughout the experimental assays) and the dispersed over continuous  
318 flow rates ratio (also constant and equal to 0.4).

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319 Membrane thickness measurements (made from pictures obtained by optical microscopy)  
320 showed a curing time-dependent thickness of alginate capsules which increased with curing  
321 time (from  $98 \pm 28$  to  $306 \pm 21$   $\mu\text{m}$  at 3 and 45 minutes, respectively) (significant differences  
322 at  $p < 0.05$ ) (see Figure 5). The increase of the membrane thickness was significant at low  
323 curing times and reached a pseudo-plateau at high curing times. The capsules reached  
324 therefore the maximum of membrane thickness after 30 min of curing time. This result was  
325 consistent with the increase of diffusion resistance and the decrease of calcium chloride  
326 concentration in time [25]. Blandino *et al.* [26] obtained nearly the same evolution for the  
327 thickness of gel layer capsules as a function of time for an alginate-CMC capsule system  
328 where thickness was measured by cutting the capsules into halves and examining the  
329 membranes with a microscope.

330 From Figures 4 and 5, it was concluded that capsule diameter and membrane thickness were  
331 linearly dependent on curing time; in addition, membrane thickness reached a plateau at high  
332 curing times due to restricted calcium diffusion. Martins *et al.* [11] checked that the emulsion  
333 drop surface was surrounded by an oily layer that limited the contact of the  $\text{CaCl}_2$  solution  
334 droplets with the external media. This external oil layer could act as a barrier hindering the  
335 release of aqueous  $\text{CaCl}_2$  droplets during the capsule production.

### 336 ***3.3.1.2. Capsules oil content***

337 In this study, droplets millifluidic was used as a rapidly expanding technology with a unique  
338 ability to “package” materials in the form of millicapsules, and a natural polysaccharide from  
339 brown seaweed as a stable container of sunflower oil. Therefore, it was very important to  
340 ascertain the capacity of the polymeric matrix to hold the bioactive compounds.

341 Oil content was around 80% for all the analyzed dried millicapsules, a content quite high  
342 compare to other encapsulation processes based on the external and on the internal gelation

343 mechanisms [27]. Once the emulsion was in contact with alginate, the process of membrane  
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2 344 formation occurred but the oil was already encapsulated. As the contribution of the membrane  
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5 345 weight was negligible with respect to the oil content, the increase in the curing time did not  
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7 346 represent a substantial change of the final core content (Figure 2, supplementary data). This  
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10 347 was the main reason why, despite the high reproducibility of the method to produce  
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12 348 monodisperse core-shell capsules, the oil content was always constant.

### 15 349 *3.3.1.3. Mechanical properties of capsules*

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19 350 Mechanical behavior is a very important characteristic, as capsules or beads have to withstand  
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21 351 mechanical stress caused by shear forces, compression or osmotic pressure, during the  
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24 352 manufacture of the food product, packaging, transport, storage, handling and consumption. In  
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26 353 particular, mechanical stiffness of alginate gel millicapsules, controlled by the strength and  
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29 354 number of cross-links between the polymer chains, influences the permeability of the  
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31 355 capsules, property highly important for solutes diffusion in cell encapsulation [28].  
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35 356 Numerous methods exist to determine the mechanical properties of capsules [29]. A well-  
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37 357 established method consists in uniaxial compression of a single capsule between two plates  
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40 358 and recording the force-displacement curve. Compression experiments are generally  
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42 359 sufficiently sensitive to characterize the mechanical behavior of liquid-filled alginate  
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45 360 capsules.

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47 361 Elastic characteristics such as Young modulus are calculated from the initial slope of the  
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50 362 force-displacement curve by means of different approaches (e.g. Hertz model) [15, 24, 29,  
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52 363 30]. The advantage of single-bead measurement consists in detailed information of a single  
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54 364 object and variations between beads of one production batch can be thoroughly analyzed.  
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57 365 Otherwise, it is time consuming to collect substantial, statistical significant data [24, 31].  
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367 Alginate capsules are sensitive to deformations that may lead to their rupture or to undesirable  
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2 368 early release of their content. It was demonstrated from the analyses of force-indentation  
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5 369 curves obtained by AFM that alginate microspheres, beads and capsules had almost a pure  
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7 370 elastic behavior [32]. Surface Young modulus, or two-dimensional Young modulus, is  
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10 371 therefore an interesting parameter to quantify mechanical stability as it describes the elastic  
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12 372 properties of the capsules. From Equation (1), compression curves at small deformations were  
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14 373 linearly fitted in order to extract the two-dimensional (or surface) Young modulus  $E_s$  under  
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17 374 point loading close to the pole [15]:

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19 375

$$22 \quad 376 \quad F = \frac{4E_s m}{a\sqrt{3(1-\nu_s^2)}} dD \quad \text{Eq. 1}$$

27 377 In this equation,  $dD$  is the capsule displacement,  $F$  the measured force,  $E_s$  the surface Young  
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29 378 modulus,  $m$  the membrane thickness,  $a$  the radius of the capsule and  $\nu_s$  the surface Poisson  
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32 379 ratio for which a value of 1/3 was assumed according to [25]. Other more complicated  
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34 380 methods of resolution by solving numeric calculations were presented in further citations [33,  
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36  
37 381 34]. It was therefore important to recall that Eq. 1 only holds for spherical capsules in the very  
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39 382 small deformations regime.

41 383 A typical force ( $F$ ) versus displacement ( $D$ ) curve from compression of single dry and wet  
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44 384 alginate capsules were shown in **Figure 6**. As expected, the drying step induced an  
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46 385 approximately ten-fold reduction of the membrane thickness due to water loss and,  
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49 386 consequently, a huge increase of the surface Young modulus was noticed (see Table 1 and  
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51 387 **Figure 6**).

54 388 Figure 7 summarized surface Young modulus values of wet alginate capsules in function of  
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56 389 curing time (calculated by linear regression from compression curves at small displacements).  
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59 390 Surface Young moduli (**significant differences at  $p < 0.05$** ) approximately two-fold decreased

391 with increasing curing time, result in apparent contradiction with the increase of membrane  
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2 392 thickness with curing time. The Surface Young modulus values were in the same order of  
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5 393 magnitude that those obtained by Leick et al. [25] for alginate capsules obtained from two  
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7 394 types of alginate with different guluronic acid content: 6.5 N/m and 32.9 N/m, for the higher  
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9 395 and lower content of guluronic acid, respectively. From another study, Leick et al. [35] found  
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11 396 surface Young moduli of 10 and 5 N/m for pure alginate and alginate/PLL capsules taking a  
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13 397 Poisson ratio of zero, values consistent with the assumption of a lower cross-linking degree of  
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15 398 the alginate/PLL composite capsules. More interestingly, each additional electrolyte  
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17 399 adsorption layer (PLL or alginate) gave roughly an increase of the surface Young modulus of  
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19 400 about 5 N/m for the investigated alginate/PLL/alginate capsule system under the used  
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21 401 preparation conditions. Messaoud et al. [36] investigated the influence of pH for alginate  
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23 402 capsules, and they obtained surface Young moduli ranging from 14.9 to 22 N/m at pH 7 and  
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25 403 pH 2, respectively, indicating that a decrease in pH resulted in a stiffer alginate membrane.  
26  
27 404 The same pH-dependence thickness of composite pectinate/shellac capsules was found by  
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29 405 Leick et al. [37]. The dependence of the pH value for composite capsules on the deformation  
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31 406 was also consistent with variations of the membrane thickness. Membranes prepared with  
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33 407 additional shellac seemed to be thinner but stiffer. Furthermore, at the lowest pH value, the  
34  
35 408 highest amount of precipitated shellac led to the strongest inhibition of calcium ion diffusion  
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37 409 and therefore to the thinnest membranes.

410 Figure 8 displayed the evolution of the surface Young modulus with the capsule dimensions.  
411 Apart from the slight decrease of surface Young modulus with the increase of wet capsule  
412 diameter (Figure 8a) and the linear decay with membrane thickness (Figure 8b), it was noticed  
413 that the surface Young modulus linearly increased with the diameter / thickness ratio of  
414 capsules (Figure 8c). Rachik et al. [14] reported that the increase in membrane thickness was  
415 linked to an increase in intrinsic membrane rigidity as it was expected that the density of

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416 crosslinking was larger in thicker membranes. Consequently, the surface Young modulus  $E_s$   
417 that measures the whole ability of the membrane to deform upon deformation increased.  
418 Contrary, in our study, surface Young modulus ranged from 68 to 21 N/m at 3 and 50 min,  
419 respectively, (see Table 1), indicating that an increase of curing time led to an increase of  
420 membrane thickness (see Figure 8b) and to a decrease of the mechanical properties (lower  
421 surface Young modulus  $E_s$  values) resulting in softer alginate membranes. This decrease of  
422 the two-dimensional modulus could be explained by a higher hydration level of the membrane  
423 leading therefore to a decrease of the crosslinking degree, i.e. the density of ~~covalent~~ ionic  
424 bonds between  $\text{Ca}^{2+}$  and alginate would be smaller in the thicker membrane due to water  
425 uptake. Following this hypothesis, ~~the~~ calcium content from the W/O droplets quickly  
426 diffused from the emulsion-core to the alginate solution and remained constant with time.  
427 This phenomenon was clearly seen on Figures 8b and 8c where a linear decrease was noticed  
428 for the surface Young modulus with the increase of membrane thickness and diameter /  
429 thickness ratio. This result therefore gave more confidence to the hypothesis of Martins et al.  
430 [11] who clearly identified by optical microscopy that the emulsion drop surface was  
431 surrounded by an oily layer that limited the contact of ~~the~~  $\text{CaCl}_2$  solution droplets with ~~the~~  
432 alginate phase. This external oil layer therefore acted as a barrier hindering the release of  
433 aqueous  $\text{CaCl}_2$  droplets during curing time.

### 434 435 **3.3.2. Effect of cation concentration**

436 In order to understand the effect of the storage solution on both membrane thickness and  
437 surface Young modulus, capsules with a curing time of 30 min in an alginate bath were stored  
438 in NaCl solution (1.5%wt) and several  $\text{CaCl}_2$  solutions at concentrations ranging from 0 to  
439 1.5%wt.



440 Microscopic observations were performed during a 48 h period to see the evolution of  
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2 441 membrane thickness (see Figure 9) (significant differences at  $p < 0.05$ ). The first important  
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4 442 observation was that membrane thickness was higher when capsules were stored in distilled  
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7 443 water contrary to  $\text{CaCl}_2$  solutions, result in agreement with the swelling of alginate membrane  
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10 444 in time. After 24 h of storage in  $\text{CaCl}_2$  solution, the membrane thickness reached a plateau  
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12 445 whatever the  $\text{CaCl}_2$  concentration (Figure 9). This result was in accordance with the absence  
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14 446 of diffusion of calcium ions from inside to the outside of the capsules and conversely  
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17 447 hindering the growth of alginate membrane in time. Finally, the storage of capsules in NaCl  
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19 448 solution led to a huge increase of membrane thickness in time, result in accordance with the  
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22 449 competition of calcium and sodium ions for alginate cross-linking and the resulting swelling  
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24 450 of alginate membrane due to the loss of calcium ions in the cross-links. The membrane  
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27 451 thickness measured at 48 h was used to evaluate the mechanical properties immediately after  
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29 452 the microscopic observations.

30  
31 453 Table 2 showed the surface Young modulus values obtained from the analyses of the force  
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34 454 displacement curves. Surface Young modulus values were constant with an average value of  
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36 455 35 N/m whatever the calcium concentration used for the storage of alginate capsules, result in  
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39 456 agreement with the constant value of membrane thickness ( $Mt \sim 360.3 \mu\text{m}$ ).

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41 457 In order to eliminate the influence of membrane thickness on the surface Young modulus and  
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44 458 to facilitate the comparison between the different storage conditions, Table 2 also showed a  
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46 459 normalized Surface Young modulus defined as  $E_s/Mt$ . Even if it was confirmed that  $E_s/Mt$   
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49 460 was constant whatever the calcium concentration used for capsules storage with  $E_s/Mt \sim$   
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51 461 0.099, it was noticed that  $E_s/Mt$  two-fold decreased when capsules were stored in distilled  
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54 462 water and more important thirty three-fold decreased when capsules were stored in NaCl. The  
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56 463 increase of membrane thickness according to the decrease of  $E_s/Mt$  was of 33% in water and  
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59 464 of 107% in NaCl. Swelling process of alginate membrane could be evoked to explain the

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465 increase of membrane thickness and therefore the decrease of surface Young modulus  
466 depending on ~~the~~ storage conditions. This swelling process would be considerably favored in  
467 presence of sodium salt compare to distilled water in particular due to the competition of  
468 sodium and calcium ions for the guluronic acid residues on alginate chains favoring the large  
469 decrease of cross-linking density in the alginate network and the huge increase of water  
470 uptake within the membrane. Authors recently clearly demonstrated, using spinning drop  
471 techniques, capsule squeezing experiments and interfacial shear rheology, changes in the  
472 mechanical properties of alginate membrane after storage of capsules in calcium or sodium  
473 salt solutions [38]. For instance, surface Young modulus determined by compression  
474 experiments decreased from 41 to 6.0 N/m when capsules were stored in calcium and sodium  
475 salt solutions, respectively. This effect was ascribed to changes of the cross-linking density  
476 and therefore a reduction of cross-linking density due to ion exchange in the case of sodium  
477 salt. It was also found recently that sugar concentrations above 15% (wt) reduced extensibility  
478 of alginate molecules and lead to a more open or less connected gel network with aggregated  
479 alginate strands [39]. In addition, authors also demonstrated the high swelling rate of alginate  
480 capsules without sugar in presence of simulated intestinal fluid, fluid containing sodium  
481 hydroxide and potassium phosphate. The effect of sodium salt could be similar to sugar or  
482 intestinal fluid by changing the alginate network and in particular pore diameter allowing a  
483 higher uptake of water in the membrane. Pore diameters ranging from 2.52 to 3.94 nm were  
484 recently reported, **using low temperature N2 adsorption-desorption analysis**, depending on  
485 alginate sample and sodium content [40].

## 487 CONCLUSIONS

488 Stable W/O emulsions with a narrow size distribution were obtained by homogenization using  
489 PGPR 90 as surfactant. Spherical millimetric capsules were successfully obtained by inverse

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490 gelation using droplets-based millifluidic. The effect of curing time and cation concentration  
491 on the oil content, structure and mechanical properties of capsules was analyzed. Alginate  
492 membrane thickness increased with curing time in the collecting alginate bath until a plateau  
493 was reached, in close relation with the presence of an oily layer surrounded the surface of  
494 W/O emulsion drops therefore limiting the contact of ~~the~~ CaCl<sub>2</sub> solution droplets with ~~the~~  
495 alginate phase. This external oil layer therefore acted as a barrier hindering the release of  
496 aqueous CaCl<sub>2</sub> droplets during curing time. Compression experiments on individual capsules  
497 revealed that alginate membrane thickness was inversely related to its mechanical properties,  
498 i.e. the thicker membrane, the lower surface Young modulus. This result was explained in  
499 terms of enhanced swelling properties of alginate membrane with curing time or storage  
500 conditions. Drying capsules led to much more resistant membranes due to the loss of water.  
501 Oil loading of 80 wt% was obtained for dry capsules whatever the conditions used.

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504 We kindly acknowledge the supply of PGPR 90 by Danisco (France), the supply of alginate  
505 by Cargill (France), the technical assistance of Jean-Eudes Megret for compression  
506 experiments, and the financial support of one author (Mariana Pereda) by the National  
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508 Exterior para Jóvenes Investigadores del CONICET” (Argentina).

510 REFERENCES

- 1  
2  
3 511 [1] Lupo, B., Maestro, A., Porras, M., Gutiérrez, J. M., & González, C. (2014). Preparation of  
4  
5 512 alginate microspheres by emulsification/internal gelation to encapsulate cocoa polyphenols.  
6  
7  
8 513 *Food Hydrocolloids*, 38, 56-65.  
9  
10 514 [2] Martins, E., Renard, D., Davy, J., Marquis, M., & Poncelet, D. (2015). Oil core  
11  
12 515 microcapsules by inverse gelation technique. *Journal of Microencapsulation*, 32(1), 86-95.  
13  
14  
15 516 [3] S.J. Risch, G.A.A. Reineccius, Flavor Encapsulation, ACS Symposium Series 370,  
16  
17 517 American Chemical Society, Washington, DC, 1988.  
18  
19  
20 518 [4] Jin, M., Zheng, Y., & Hu, Q. (2009). Preparation and characterization of bovine serum  
21  
22 519 albumin alginate/chitosan microspheres for oral administration. *Asian Journal of*  
23  
24 520 *Pharmaceutical Sciences*, 4(4), 215-220.  
25  
26  
27 521 [5] Ziani, K., Fang, Y., & McClements, D. J. (2012). Encapsulation of functional lipophilic  
28  
29 522 components in surfactant-based colloidal delivery systems: vitamin E, vitamin D, and lemon  
30  
31 523 oil. *Food chemistry*, 134(2), 1106-1112.  
32  
33  
34 524 [6] Ouwerx, C., Velings, N., Mestdagh, M. M., & Axelos, M. A. V. (1998). Physico-chemical  
35  
36 525 properties and rheology of alginate gel beads formed with various divalent cations. *Polymer*  
37  
38 526 *Gels and Networks*, 6(5), 393-408.  
39  
40  
41 527 [7] Poncelet, D., Babak, V., Dulieu, C., & Picot, A. (1999). A physico-chemical approach to  
42  
43 528 production of alginate beads by emulsification-internal ionotropic gelation. *Colloids and*  
44  
45 529 *Surfaces A: Physicochemical and Engineering Aspects*, 155(2-3), 171-176.  
46  
47  
48  
49 530 [8] Quong, D., Neufeld, R. J., Skjåk- Bræk, G., & Poncelet, D. (1998). External versus  
50  
51 531 internal source of calcium during the gelation of alginate beads for DNA encapsulation.  
52  
53 532 *Biotechnology and Bioengineering*, 57(4), 438-446.  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 533 [9] Abang, S., Chan, E. S., & Poncelet, D. (2012). Effects of process variables on the  
1 encapsulation of oil in ca-alginate capsules using an inverse gelation technique. *Journal of*  
2 534 *microencapsulation*, 29(5), 417-428.  
3  
4  
5 535  
6  
7 536 [10] Martins, E., Poncelet, D., & Renard, D. (2017a). A novel method of oil encapsulation in  
8  
9  
10 537 core-shell alginate microcapsules by dispersion-inverse gelation technique. *Reactive and*  
11  
12 538 *Functional Polymers*, 114, 49-57.  
13  
14 539 [11] Martins, E., Poncelet, D., Marquis, M., Davy, J., & Renard, D. (2017b). Monodisperse  
15  
16 540 core-shell alginate (micro)-capsules with oil core generated from droplets millifluidic. *Food*  
17  
18 541 *Hydrocolloids*, 63, 447-456.  
19  
20  
21 542 [12] Wang, J-Y., Jin, Y., Xie, R., Liu, J-Y., Ju, X-J., Meng, T., & Chu, L-Y. (2011). Novel  
22  
23 543 calcium-alginate capsules with aqueous core and thermo-responsive membrane. *Journal of*  
24  
25 544 *Colloid and Interface Science*, 353, 61-68.  
26  
27  
28 545 [13] Schmit, A., Courbin, L., Marquis, M., Renard, D., & Panizza, P. (2014). A pendant drop  
29  
30 546 method for the production of calibrated double emulsions and emulsion gels. *Rsc Advances*,  
31  
32 547 4(54), 28504-28510.  
33  
34  
35 548 [14] Rachik, M., Barthes-Biesel, D., Carin, M., & Edwards-Levy, F. (2006). Identification of  
36  
37 549 the elastic properties of an artificial capsule membrane with the compression test: effect of  
38  
39 550 thickness. *Journal of colloid and interface science*, 301(1), 217-226.  
40  
41  
42 551 [15] Fery, A., & Weinkamer, R. (2007). Mechanical properties of micro-and nanocapsules:  
43  
44 552 Single-capsule measurements. *Polymer*, 48(25), 7221-7235.  
45  
46  
47 553 [16] Al-Sabagh, A. M. (2002). The relevance HLB of surfactants on the stability of asphalt  
48  
49 554 emulsion. *Colloids and Surfaces a-Physicochemical and Engineering Aspects*, 204(1-3), 73-  
50  
51 555 83.  
52  
53  
54 556 [17] Márquez, A. L., Medrano, A., Panizzolo, L. A., & Wagner, J. R. (2010). Effect of  
55  
56 557 calcium salts and surfactant concentration on the stability of water-in-oil (w/o) emulsions  
57  
58  
59  
60  
61  
62  
63  
64  
65

558 prepared with polyglycerol polyricinoleate. *Journal of colloid and interface science*, 341(1),  
1  
2 559 101-108.  
3  
4  
5 560 [18] Su, J. H., Flanagan, J., Hemar, Y., & Singh, H. (2006). Synergistic effects of  
6  
7 561 polyglycerol ester of polyricinoleic acid and sodium caseinate on the stabilisation of water-  
8  
9 562 oil-water emulsions. *Food Hydrocolloids*, 20(2-3), 261-268.  
10  
11  
12 563 [19] Karunadasa, K. S., Manoratne, C. H., Pitawala, H. M. T. G. A., & Rajapakse, R. M. G.  
13  
14 564 (2018). Relative stability of hydrated/anhydrous products of calcium chloride during complete  
15  
16 565 dehydration as examined by high-temperature X-ray powder diffraction. *Journal of Physics*  
17  
18 566 and Chemistry of Solids, 120, 167-172.  
19  
20  
21 567 [20] Souza, A., Santos, J. C., Conceição, M. M., Silva, M. C., & Prasad, S. (2004). A  
22  
23 568 thermoanalytic and kinetic study of sunflower oil. *Brazilian Journal of Chemical Engineering*,  
24  
25 569 21(2), 265-273.  
26  
27  
28 570 [21] Zhao, Y., Huang, Z., Zhang, J., Wu, W., Wang, M., & Fan, L. (2010). Thermal  
29  
30 571 Degradation of Sodium Alginate-Incorporated Soy Protein Isolate/Glycerol Composite  
31  
32 572 Membranes.  
33  
34  
35 573 [22] Soares, J. P., Santos, J. E., Chierice, G. O., & Cavalheiro, E. T. G. (2004). Thermal  
36  
37 574 behavior of alginic acid and its sodium salt. *Eclética Química*, 29(2), 57-64.  
38  
39  
40 575 [23] Pawlik, A. K. (2012). Duplex emulsions for healthy foods (Doctoral dissertation,  
41  
42 576 University of Birmingham).  
43  
44  
45 577 [24] Gray, A., Egan, S., Bakalis, S., & Zhang, Z. (2016). Determination of microcapsule  
46  
47 578 physicochemical, structural, and mechanical properties. *Particuology*, 24, 32-43.  
48  
49  
50 579 [25] Leick, S., Henning, S., Degen, P., Suter, D., & Rehage, H. (2010). Deformation of liquid-  
51  
52 580 filled calcium alginate capsules in a spinning drop apparatus. *Physical Chemistry Chemical*  
53  
54 581 *Physics*, 12(12), 2950-2958.  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 582 [26] Blandino, A., Macias, M., & Cantero, D. (1999). Formation of calcium alginate gel  
1 capsules: influence of sodium alginate and CaCl<sub>2</sub> concentration on gelation kinetics. Journal  
2 583 of bioscience and bioengineering, 88(6), 686-689.  
3  
4 584
- 585 [27] Martins, E., Poncelet, D., Ramires, C.R., & Renard, D. (2017c). Oil encapsulation  
6  
7 585 techniques using alginate as encapsulating agent: Applications and drawbacks. Journal of  
8  
9 586  
10 Microencapsulation, 34 (8) 754-771.  
11  
12 587
- 588 [28] Briššová, M., Lacík, I., Powers, A.C., Anilkumar, A.V., & Wang, T. (1998). Control and  
13  
14 588 measurement of permeability for design of microcapsule cell delivery system. Journal of  
15  
16 589  
17 Biomedical Materials Research, 39(1), 61-70.  
18  
19 590
- 591 [29] Neubauer, M. P., Poehlmann, M., & Fery, A. (2014). Microcapsule mechanics: From  
20  
21 591  
22 stability to function. Advances in colloid and interface science, 207, 65-80.  
23  
24 592
- 593 [30] Chan, E. S., Lim, T. K., Voo, W. P., Pogaku, R., Tey, B. T., & Zhang, Z. (2011). Effect  
25  
26 593  
27 of formulation of alginate beads on their mechanical behavior and stiffness. Particuology,  
28  
29 594  
30 9(3), 228-234.  
31  
32 595
- 596 [31] Marcadé-Prieto, Z. Zhang Mechanical characterization of microspheres - capsules, cells  
33  
34 596  
35 and microspheres: A review Journal of Microencapsulation, 29 (3) (2012), pp. 277-285.  
36  
37 597
- 598 [32] Lekka, M., Sainz-Serp, D., Kulik, A.J., & Wandrey, C. (2004). Hydrogel Microspheres:  
38  
39 598  
40 Influence of Chemical Composition on Surface Morphology, Local Elastic Properties, and  
41  
42 599  
43 Bulk Mechanical Characteristics. Langmuir, 20, 9968-9977.  
44  
45 600
- 601 [33] Carin, M., Barthès- Biesel, D., Edwards- Lévy, F., Postel, C., & Andrei, D. C. (2003).  
46  
47 601  
48 Compression of biocompatible liquid- filled HSA- alginate capsules: Determination of the  
49  
50 602  
51 membrane mechanical properties. Biotechnology and bioengineering, 82(2), 207-212.  
52  
53 603
- 604 [34] Keller, M. W., & Sottos, N. R. (2006). Mechanical properties of microcapsules used in a  
54  
55 604  
56 self-healing polymer. Experimental Mechanics, 46(6), 725-733.  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 606 [35] Leick, S., Kemper, A., & Rehage, H. (2011). Alginate/poly-L-lysine capsules:  
1  
2 607 mechanical properties and drug release characteristics. *Soft Matter*, 7, 6684-6694.  
3  
4  
5 608 [36] Messaoud, G. B., Sánchez-González, L., Jacquot, A., Probst, L., & Desobry, S. (2015).  
6  
7 609 Alginate/sodium caseinate aqueous-core capsules: A pH-responsive matrix. *Journal of colloid*  
8  
9 610 and interface science, 440, 1-8.  
10  
11  
12 611 [37] Leick, S., Kott, M., Degen, P., Henning, S., Päsler, T., Suter, D., & Rehage, H. (2011).  
13  
14 612 Mechanical properties of liquid-filled shellac composite capsules. *Physical Chemistry*  
15  
16 613 *Chemical Physics*, 13(7), 2765-2773.  
17  
18  
19 614 [38] Zwar, E., Kemna, A., Richter, L., Degen, P., & Rehage, H. (2018). Production,  
20  
21 615 deformation and mechanical investigation of magnetic alginate capsules. *Journal of Physics-*  
22  
23 616 *Condensed Matter*, 30(8), number 085101.  
24  
25  
26 617 [39] Lopez-Sanchez, P., Fredriksson, N., Larsson, A., Altskärc, A., & Strömb, A. (2018).  
27  
28 618 High sugar content impacts microstructure, mechanics and release of calcium-alginate gels.  
29  
30 619 *Food Hydrocolloids*, 84, 26-33.  
31  
32  
33  
34 620 [40] Ramos, P.E., Silva, P., Alario, M.M., Pastrana, L.M., Teixeira, J.A., Cerqueira, M.A.,  
35  
36 621 & Vicente, A.A. (2018). Effect of alginate molecular weight and M/G ratio in beads properties  
37  
38 622 foreseeing the protection of probiotics. *Food Hydrocolloids*, 77, 8-16.  
39  
40  
41 623  
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43 624  
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631 **Table 1.** Mechanical properties of wet and dry capsules. Curing time = 30 min.

632 conditions.

	<b>Diameter</b> <b>D</b> <b>(mm)</b>	<b>Membrane</b> <b>thickness</b> <b>Mt</b> <b>(<math>\mu\text{m}</math>)</b>	<b>D/Mt</b>	<b>Surface Young</b> <b>modulus</b> <b>(N/m)</b>	<b>Force at</b> <b>break</b> <b>(N)</b>
Wet capsules	2.21± 0.07	297± 76	7.4± 0.3	26.1± 1.8	1.15± 0.27
Dry capsules	1.85± 0.14	27± 12*	60.1± 5.9	613± 163	1.45± 0.37

633 \*Membrane thickness was estimated from other capsules that were obtained and dried in the same

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635 Table 2. Surface Young modulus values of capsules after 48h storage in distilled water, CaCl<sub>2</sub> or NaCl  
 636 solutions. Curing time in alginate solution = 30 min.  
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708	709	710	711	712	713	714
715	716	717	718	719	720	721
722	723	724	725	726	727	728
729	730	731	732	733	734	735
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764	765	766	767	768	769	770
771	772	773	774	775	776	777
778	779	780	781	782	783	784
785	786	787	788	789	790	791
792	793	794	795	796	797	798
799	800	801	802	803	804	805
806	807	808	809	810	811	812
813	814	815	816	817	818	819
820	821	822	823	824	825	826
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848	849	850	851	852	853	854
855	856	857	858	859	860	861
862	863	864	865	866	867	868
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932	933	934	935	936	937	938
939	940	941	942	943	944	945
946	947	948	949	950	951	952
953	954	955	956	957	958	959
960	961	962	963	964	965	966
967	968	969	970	971	972	973
974	975	976	977	978	979	980
981	982	983	984	985	986	987
988	989	990	991	992	993	994
995	996	997	998	999	1000	
Distilled Water	2.27 ± 0.20 <sup>a,c</sup>	479 ± 74 <sup>a</sup>	4.7 ± 0.3 <sup>a,d</sup>	23.4 ± 2.64 <sup>a</sup>	0.049	
CaCl <sub>2</sub> 0.1	2.43 ± 0.17 <sup>a,b,c</sup>	388 ± 84 <sup>a,b,c</sup>	6.3 ± 1.7 <sup>a,b,c</sup>	28.7 ± 4.6 <sup>a</sup>	0.073	
CaCl <sub>2</sub> 0.3	2.33 ± 0.18 <sup>a,b,c</sup>	316 ± 44 <sup>b,c</sup>	7.4 ± 1.4 <sup>b,c</sup>	39.5 ± 3.3 <sup>b,c</sup>	0.125	
CaCl <sub>2</sub> 1	2.40 ± 0.19 <sup>a,b,c</sup>	392 ± 61 <sup>a,b,c</sup>	6.1 ± 1.1 <sup>a,b,c</sup>	29.1 ± 6.6 <sup>a,b</sup>	0.074	
CaCl <sub>2</sub> 1.5	2.60 ± 0.14 <sup>b</sup>	345 ± 48 <sup>c</sup>	7.5 ± 1.1 <sup>c</sup>	42.6 ± 6.9 <sup>c</sup>	0.123	
NaCl 1.5	2.11 ± 0.14 <sup>c</sup>	748 ± 96 <sup>d</sup>	2.8 ± 0.5 <sup>d</sup>	2.3 ± 0.1 <sup>d</sup>	0.003	

638 a,b,c,d Different letters in the same column indicate significant differences among the different storage solutions (p  
 639 < 0.05).

685 **Figure captions**

1 686

3 687 Figure 1. Images of W/O emulsions as function of storage time.

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7 689 Figure 2. Micrographs of a) oil diluted W/O emulsion (scale bar 25  $\mu\text{m}$ ) and b) water diluted W/O  
8 690 emulsion (scale bar 250  $\mu\text{m}$ ) resulting in the formation of a double W/O/W emulsion.

10 691

11 692 Figure 3. Thermogravimetric analyses of sunflower oil, alginate and calcium chloride powders used to  
12 693 prepare capsules.

14 694

16 695 Figure 4. Micrographs of wet capsules as function of curing time. Scale bar 250  $\mu\text{m}$ .

18 696

19 697 Figure 5. Diameter  $d$  (mm) and membrane thickness  $Mt$  ( $\mu\text{m}$ ) of wet capsules as function of curing  
20 698 time. **Solid line corresponded to the fitting of the data by a linear function; dotted line was just eye-**  
21 699 **guide. At the 0.05 level, the populations means were significantly different.**

24 700

26 701 Figure 6. Compression behavior of wet and dry capsules. Curing time = 30 min.

28 702

29 703 Figure 7. **Surface Young modulus (N/m)** of wet capsules as function of curing time. **At the 0.05 level,**  
30 704 **the populations means were significantly different.**

32 705

34 706 Figure 8. Evolution of the surface Young modulus with wet capsule dimensions: a) diameter; b)  
35 707 membrane thickness and c) diameter/thickness ratio.

37 708

38 709 Figure 9. Evolution of the membrane thickness after different storage times in distilled water,  $\text{CaCl}_2$  or  
39 710  $\text{NaCl}$  solutions. Curing time in alginate solution = 30 min. **At the 0.05 level, the populations means**  
40 711 **were significantly different.**

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Figure 1

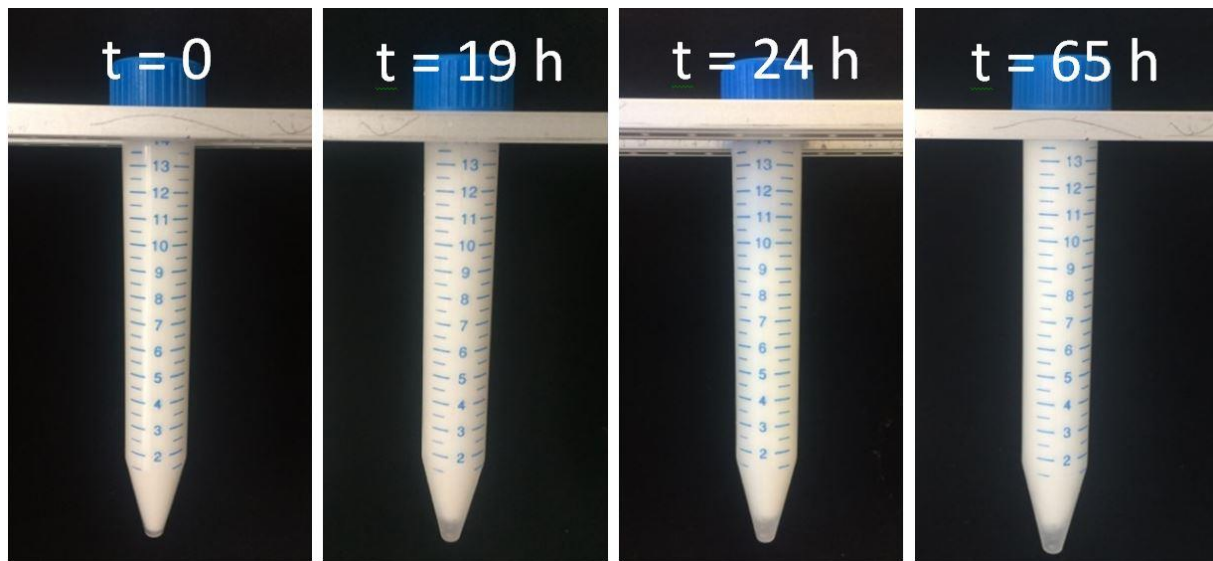


Figure 2

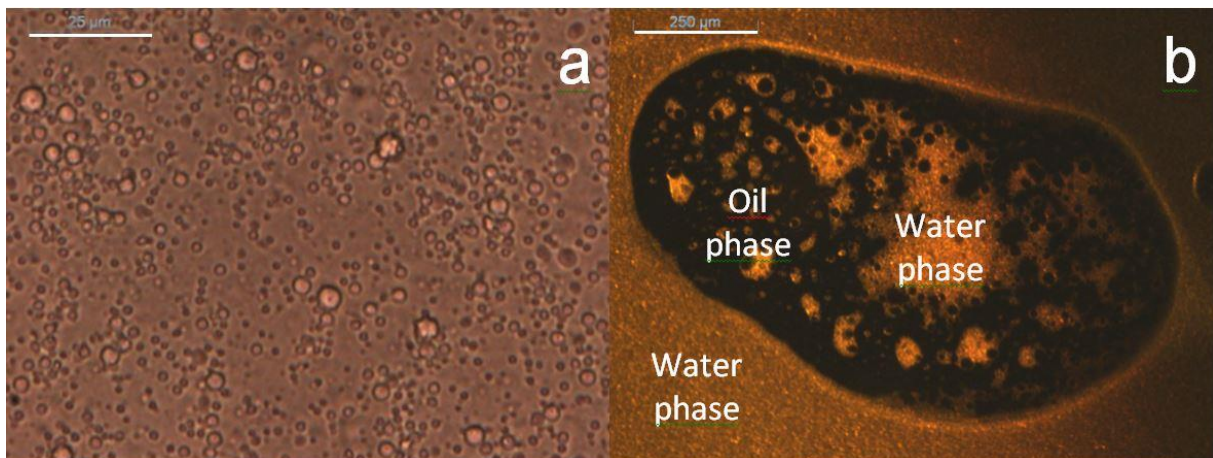


Figure 3 (revised)

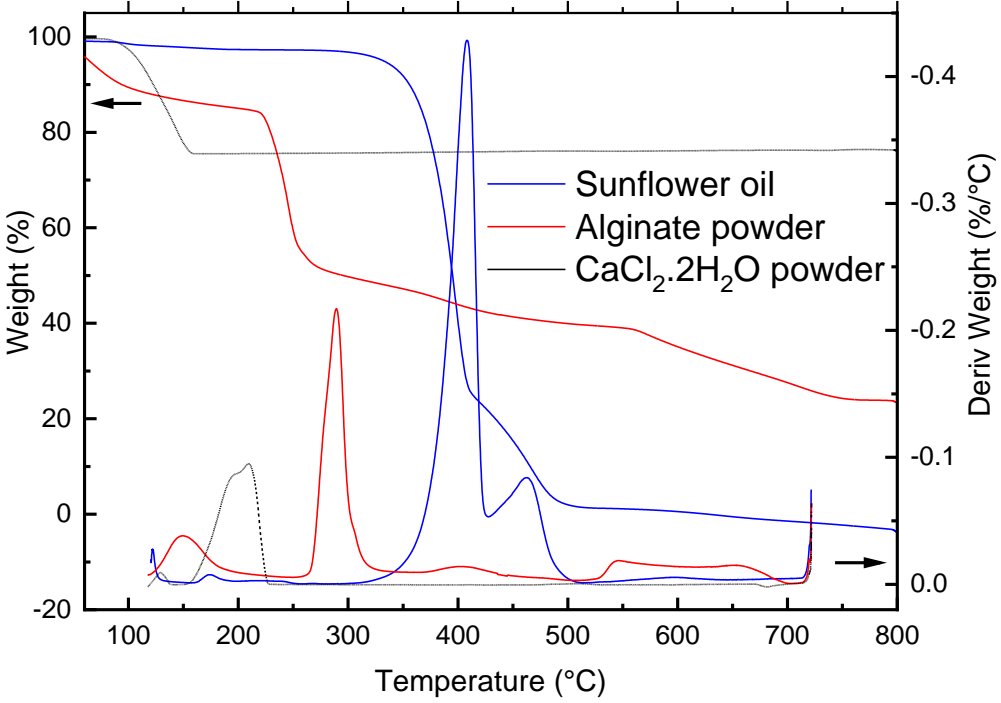


Figure 4 (revised)

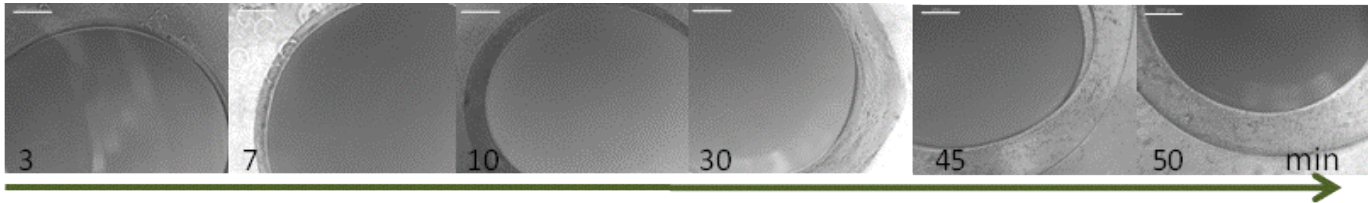


Figure 5 (revised)

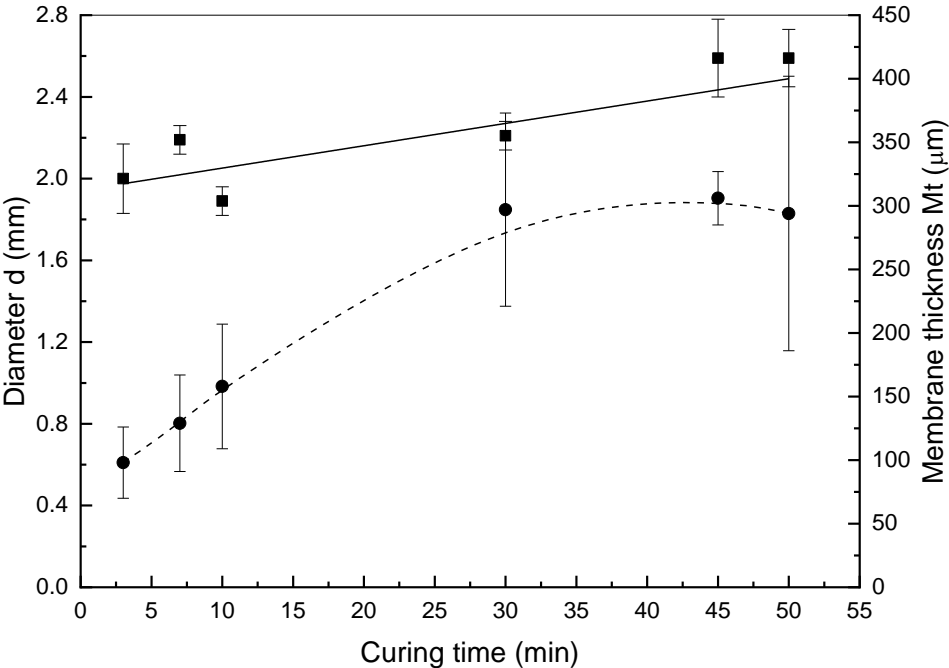


Figure 6

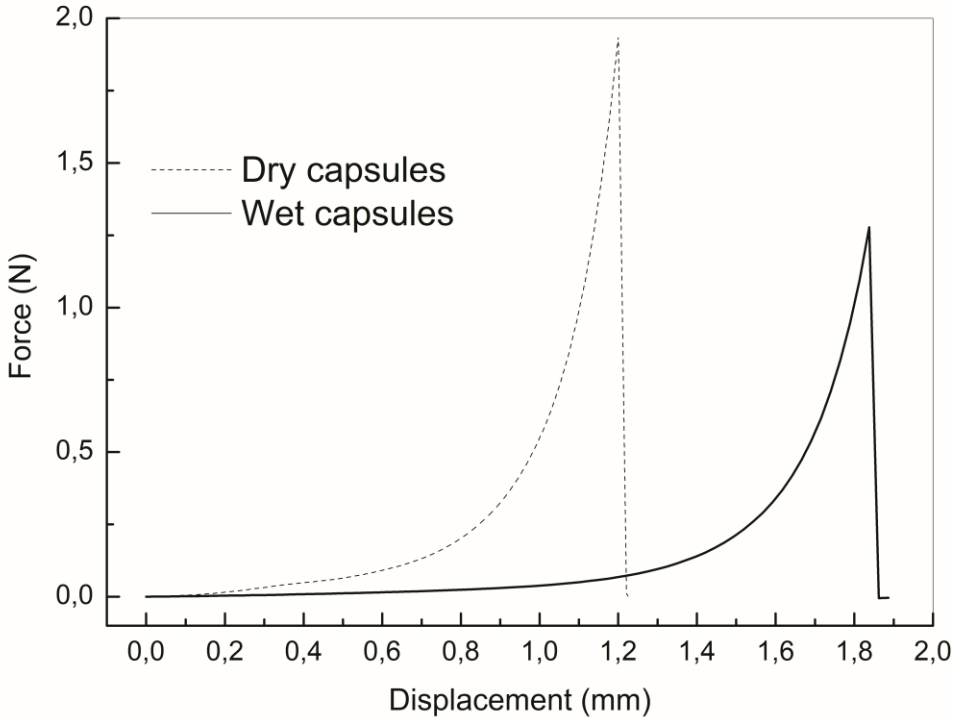


Figure 7 (revised)

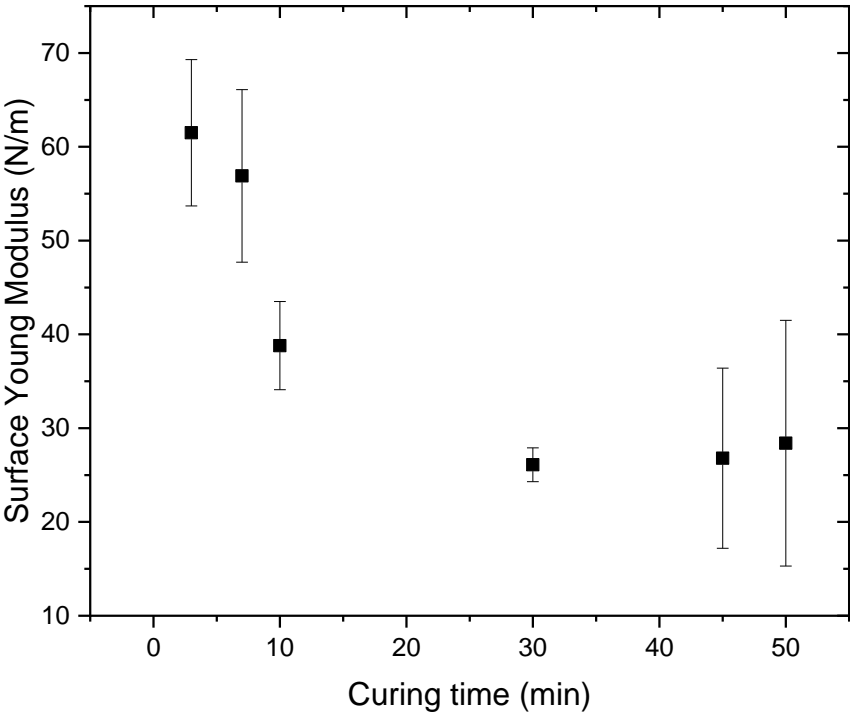




Figure 8 (revised)

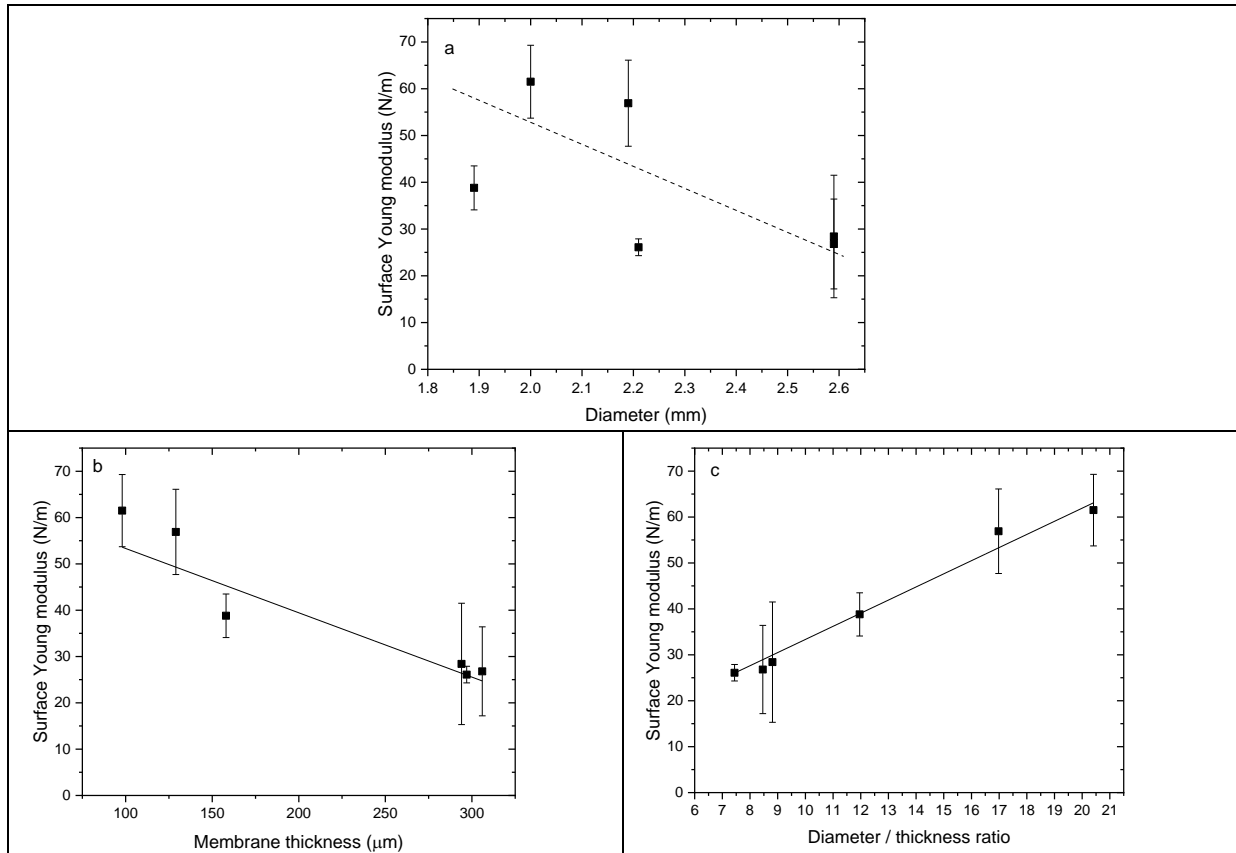
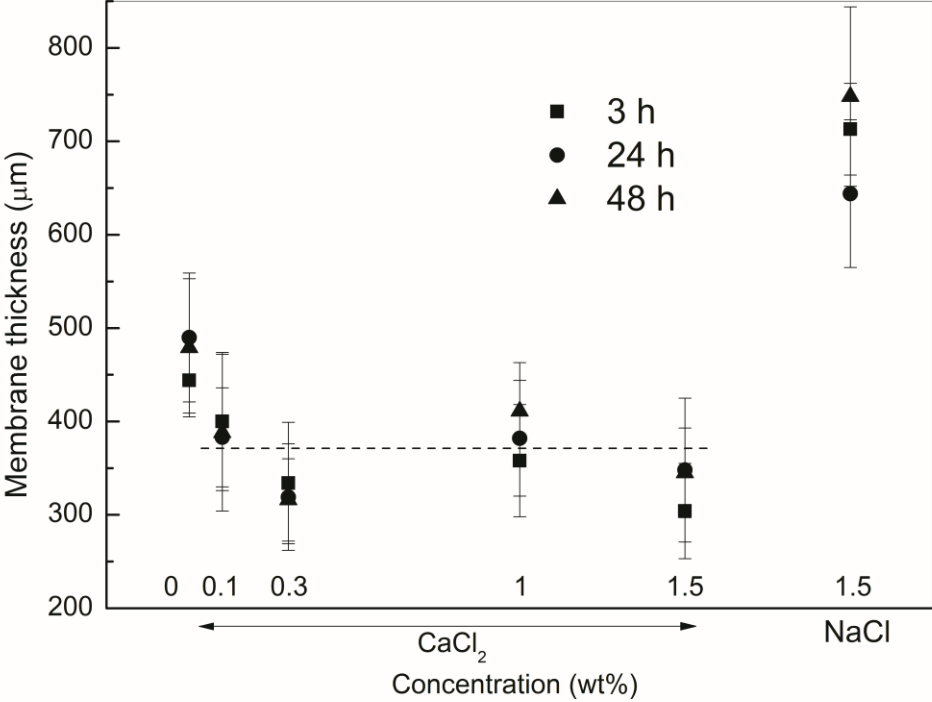


Figure 9





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**Supplementary Material**

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