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**Title:** *Advances on Alginate use for spherification to encapsulate biomolecules.*

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**Abstract:** *Spherification methods to produce particles of millimetric size are detailed in this review, especially for encapsulation purpose and using alginate. Therefore, alginate properties and factors impacting its gelation, counting its structure and experimental conditions such as pH, temperature, crosslinker concentration, residence time and ionic strength, are largely discussed. Furthermore, the interest for using co-polymer in spherification is investigated. Thereby, alginate composite gels can enhance encapsulation efficiency, mechanical stability, barrier properties and thermal stability. The advantages and limitations of their use are discussed for each spherification process to improve the knowledge about alginate versatility. Alginate is still one of the most promising encapsulation materials.*

**Keywords:**

*Alginate, Hydrocolloids, Spherification, Encapsulation, Composite, Coextrusion.*

1        1. Introduction

2 Alginate is the salt of alginic acid, a natural polymer of uronic acids (mannuronic and  
3 guluronic acids) extracted especially from brown marine algae (Blanco-Cabra et al., 2020;  
4 Boyd & Chakrabarty, 1995; Günter et al., 2020) and also from some bacteria, such as  
5 *Pseudomonas aeruginosa*.

6 The alginate gelation is one common way to produce beads or capsules. The term  
7 spherification used in this review covers all the methods employed to design such particles of  
8 millimetric size, ideally spherical. In this review, we consider spherification with the aim of  
9 encapsulating different ingredients and active principles.

10 Alginate is Generally Recognized as Safe (GRAS), being nontoxic, nonantigenic,  
11 satisfactorily biocompatible, and sufficiently biodegradable (Lee & Mooney, 2012). As a  
12 result of its physicochemical properties, alginate is used for a large variety of technical  
13 applications, as a coating material in textile industry (Misbah et al., 2019) or as a  
14 disintegrating agent for tablets. Alginate is also commonly found in cosmetic (Adli et al.,  
15 2020), pharmaceutical, medical (Shi et al., 2020) and food industries, as thickener and  
16 stabilizer (Senturk Parreidt et al., 2018; C. Su et al., 2018; H. Zhang et al., 2019).

17 **Table 1**

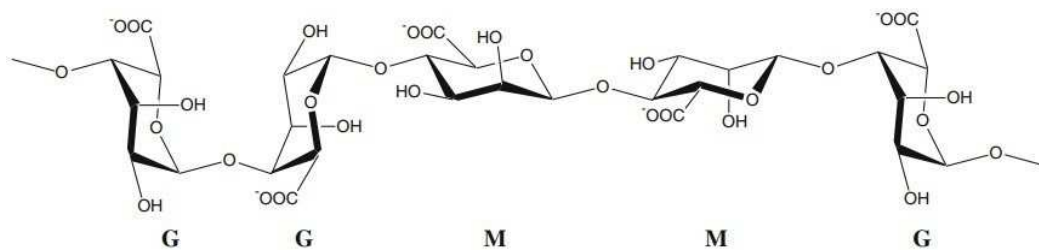
18 Alginates types and their applications

Code	Alginate types	Applications	References
E400	Alginic acid	Zero calorie ingredients for diet foods (biscuits, low-sugar marmalade, ice-creams).	Mancini & McHugh, 2013.
E401	Sodium alginate	Stabilizer and thickener in frostings,	Norton et al., 2010; Qin et
E402	Potassium alginate	puddings and processed fruits and fruit	al., 2018.
E403	Ammonium alginate	juices.	
E404	Calcium alginate	Matrix for encapsulation of volatiles constituents. Coating films to preserve frozen fish. Pearls from spherification process.	
E405	Propane-1,2 diol alginate	Stabilizer of emulsion in acidic environment (salad dressing). Stabilizer of foam in brewing industry.	Norton et al., 2010.

20 A major use of alginate today is the encapsulation technology through its biocompatibility and  
21 capability to form a hydrogel through ionic cross-links with divalent cations, such as calcium  
22 ions (B. B. Lee et al., 2016; Martău et al., 2019; Ramos et al., 2018).

23 1.1. Alginate molecular structure and its modification by processing methods

24 Alginate is formed by a chain of  $\beta$ -D-mannuronic acid (M units) and  $\alpha$ -L-guluronic acid (G  
25 units), linked via (1 $\rightarrow$ 4) glycosidic linkage. The percentage of those units varies among algae  
26 species (Rhein-Knudsen et al., 2017) and impacts alginate properties (Hecht & Srebnik,  
27 2016).



28  
29 **Fig. 1.** Structural characteristics of alginate. M: mannuronate residue; G: guluronate residue.

30 As polysaccharides properties are tightly related to their structures, the modification of  
31 alginate structure has been widely explored. For that, alginate acid hydrolysis showed a uronic  
32 acids release through an elimination reaction that led to molecular weight reduction (Haug,  
33 Larsen, Smidsröd, et al., 1963; J. S. Yang et al., 2011). Chhatbar et al. (2009) studied the  
34 effect of microwave-assisted acidic hydrolysis of alginate, and reported an acceleration of this  
35 reaction with similar oligo-alginate properties than conventional acidic hydrolysis.

36 Alginate can also be enzymatically and thermally treated (Aida et al., 2010; Hartmann et al.,  
37 2006; Kelishomi et al., 2016) to modify its structure and functional properties through the  
38 molecular weight reduction or M/G ratio modification. The enrichment with G units results in  
39 a rigid structure, when an enrichment with M units produces a flexible molecular structure  
40 (Vold et al., 2006).

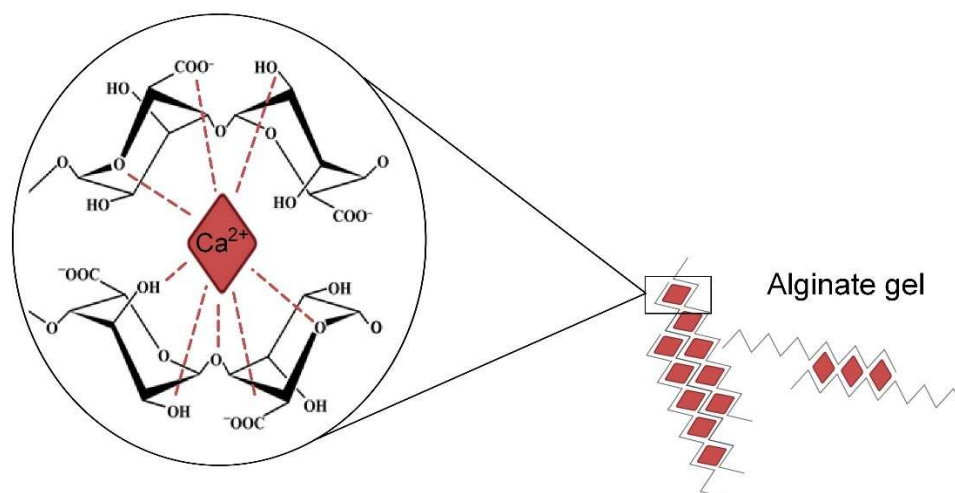
41 Ultrasounds treatment of alginate at different frequencies induce polymer structure  
42 degradation, rearrangements and modifications of its molecular weight. A decrease of M/G  
43 ratio has also been reported with changes of hydrophobic interactions making alginate treated  
44 by 135 kHz having a stronger stiffness compared to the untreated polymer (Feng et al., 2017;  
45 Taha et al., 2020).

46 In addition to their physicochemical properties, biological activities of oligo-alginate obtained  
47 by alginate acidic hydrolysis have been investigated. Plant growth bio-stimulating activity  
48 was demonstrated (Salachna, et al., 2018). Anti-allergic activity of a 13.3 kDa oligo-alginate  
49 was reported after scission of an initial 1 605 kDa alginate polymer by a 3kGy alpha-  
50 irradiation (Eu, et al 2008).

### 51 1.2. Ion-binding for alginate gelation

52 One of the most studied properties of alginate, is its ability to form hydrogels by cross-  
53 linking with divalent or trivalent cations such as  $\text{Ca}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Al}^{3+}$ , for example. Those  
54 cations present various affinities toward alginate, that result in hydrogels with different  
55 structural and mechanical characteristics (Young modulus, gel morphology, etc) (Agulhon et  
56 al., 2012; Kabir et al., 2020; Vinet & Zhedanov, 2011).

57 For food or cosmetic application,  $\text{Ca}^{2+}$  is the most commonly used. So, in the literature,  
58 calcium alginate gels are the most largely investigated. Gelation occurs in solution when  
59 calcium ions replace sodium to cross-link two alginate chains and form a network (Vinet &  
60 Zhedanov, 2011; Warsinger et al., 2015). Alginate gelation is commonly described as an  
61 eggbox model formed by two antiparallel chains of polyuronates linked with  $\text{Ca}^{2+}$  binding  
62 blocks as illustrated in fig.2 (Martău et al., 2019; Reynolds & Enquist, 2006). Stokke et al.  
63 (2000) suggested a lateral association of G units in the eggbox structure.



64  
65 **Fig. 2.** Alginate gel structure in presence of calcium ions (egg-box model) (Adapted from  
66 Martău et al., 2019)

## 67 2. Spherification process

### 68 2.1. Gelation methods

69 Two main gelation methods can be presented: (i) the external gelation, also called diffusion  
70 process (Bajpai et al., 2016; Rezvanain et al., 2017); and (ii) the internal gelation also called  
71 pH-controlled gelation as pH determines gelation kinetics (Cao et al., 2020; Hernández-  
72 González et al., 2020). These two methods are described below.

#### 73 2.1.1. External gelation

74 The external gelation method leads to gel formation through diffusion of calcium ions in an  
75 alginate solution (X. D. Liu et al., 2002). Alginate solution droplets are usually extruded into  
76 a calcium bath; this method is called conventional or classical spherification. A solution  
77 containing calcium ions can also be dropped into an alginate bath and this process is known as  
78 inverse spherification.

79 Gel prepared by external gelation presents an inhomogeneous structure with low mechanical  
80 properties, this is due to the presence of calcium ions mostly at the surface (Arriola et al.,  
81 2019; McClements, 2017; Spadari et al., 2019; Q. Sun et al., 2018). A concentration gradient  
82 of polymer/ions exists from beads surface to core (Sagdicoglu Celep et al., 2020). Several  
83 researches focused on this feature to overcome it (Hernández-González et al., 2020; Puguan et  
84 al., 2014), or to study bioactive substances delivery systems in food and medicine applications  
85 (X. D. Liu et al., 2002; Mancini & McHugh, 2000).

#### 86 2.1.2. Internal gelation

87 Internal gelation method is based on calcium release simultaneously into the all system under  
88 controlled conditions. Usually calcium lactate is dissociated to initiate alginate gelation  
89 (Strobel et al., 2020). Internal gelation allows a better control of gelation kinetics and  
90 produces a homogeneous gel structure with pores. It is used for controlled delivery of active  
91 compounds (Hernández-González et al., 2020). As an example, Strobel et al. (2020) reported  
92 a significative enhance of fish oil stability after its encapsulation with internal gelation  
93 method.

94 Javvaji et al. (2011) reported a novel light activated gelation method induced by photoacid  
95 generation in an insoluble calcium salt dispersion. After 10min of UV-treatment, the released  
96 H<sup>+</sup> ions from the photoacid generator react with the calcium salt and release calcium ions that  
97 cross linked with alginate to form a hydrogel.

98

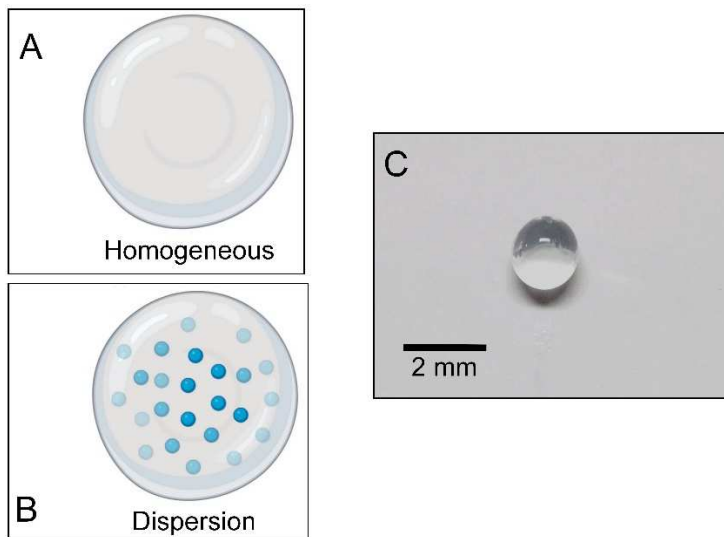
99 2.2. Spherification process using alginate

100 Encapsulation process permits to coat a substance for its preservation and/or addition of  
101 functional properties. Several encapsulation methods involving alginate can give rise to  
102 particles or capsules with several structural organizations. Thus, many parameters have to be  
103 considered to choose the appropriate particle structure associated to an encapsulation method:  
104 particle size, composition of encapsulated substance, stability of matrix and delivery features.  
105 Besides, those methods have to be scaled up for industrial production (Ching et al., 2017).

106 2.2.1. Extrusion

107 2.2.1.1. Simple dripping

108 Simple extrusion process is the most commonly spread encapsulation way. The principle  
109 consists in extrusion through a nozzle of an alginate solution or an emulsion containing  
110 alginate. The formed droplets fall into a gelation bath containing an alginate crosslinker  
111 (generally  $\text{Ca}^{2+}$ ) under stirring. Following this basic principle, the process can be varied to  
112 incorporate interesting components into alginate solution/emulsion in order to encapsulate it  
113 and trap it into the matrix (Ching et al., 2017). Different structural organization can be  
114 produced depending on the extruded solution. For instance, an alginate solution loaded with  
115 the target compound tends to produce beads with homogenous microstructure Fig.3A. An  
116 emulsion consisting in a lipidic phase dispersed in an aqueous phase of alginate results in  
117 obtention of beads with a dispersed microstructure Fig.3B.



119 **Fig. 3.** Scheme of homogeneous structural organization for capsules (A), scheme of dispersed  
120 structural organization for capsules (B) and a photograph of a homogenous capsule.

121 The beads shape depends on experimental conditions such as pH, calcium concentration and  
122 temperature. However, beads sphericity is also determined by alginate solution viscosity and  
123 fall distance between nozzle outlet and bath surface. The optimized fall distance to obtain  
124 spherical beads has been determined in the range 7 to 10 cm knowing that droplets are formed  
125 as a result of solution surface tension. This distance is necessary to the liquid to drag the  
126 forces exerted by the gelling bath and overcome the impact to form a spherical bead. Also,  
127 sphericity can be improved through surface active compounds addition (Blandino et al., 1999;  
128 Ching et al., 2017)

129 Simple extrusion process allowed encapsulation of various compounds for delivery systems  
130 and to increase their stability, including oils (Bannikova et al., 2018), essential oils  
131 (Benavides et al., 2016; Gholamian et al., 2021), drugs (Hariyadi & Islam, 2020), probiotics  
132 (Dehkordi et al., 2020; Praepanitchai et al., 2019), enzymes (Kurayama et al., 2020) and  
133 proteins (Y. Su et al., 2019).

134 In order to produce particles of different sizes (from 20 $\mu$ m to 7mm), numerous encapsulation  
135 processes have been derived from simple dripping process. Those methods differ generally by  
136 the ways of formation and separation of alginate droplets. Usually, these methods are divided  
137 into three groups: jet dispersion, spinning disk and atomization.

138 • Jet dispersion

139 The extruded polymer gel is split up following different methods. In this group, vibrating  
140 nozzle (Pedrali et al., 2020), electrostatic atomization (Nikoo et al., 2018) and jet cutting  
141 (Paulo et al., 2017) are commonly used. It is also possible to merge particular processes for  
142 increased efficiency, as the vibrating electrostatic method for example (Pedrali et al., 2020).

143 • Spinning disk

144 Under centrifugal force, the alginate solution flow forms droplets due to the rotation of the  
145 nozzle (or rotating disc). Spinning disk method is used to encapsulate flavor ingredients (Yin  
146 & Cadwallader, 2018).

147 • Atomization

148 Another extrusion method is atomization. The process basis is the extrusion of pressurized  
149 polymer solution through a nozzle while injecting pressurized air, atomized particles fall then

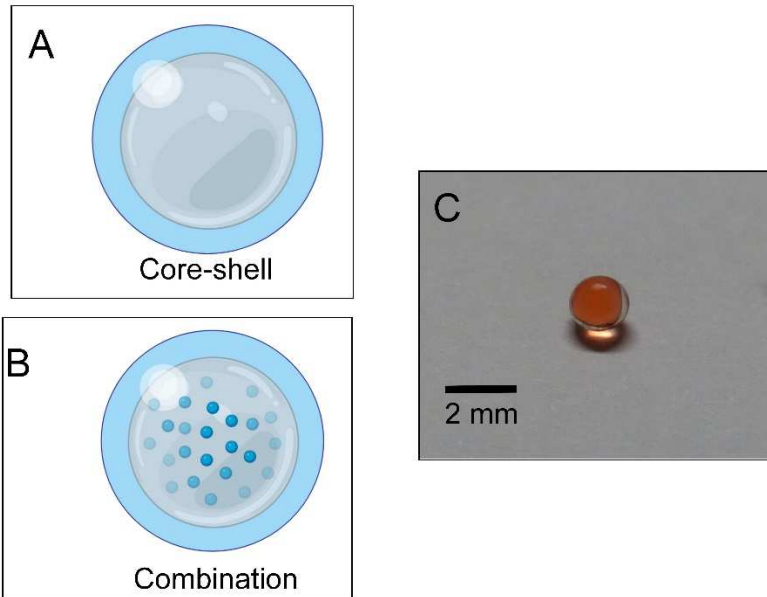


150 into a gelling bath. Atomization can be applied for lipid encapsulation for example (Strasdat  
151 & Bunjes, 2013).

### 152 2.2.2. Coextrusion

153 Coextrusion, also called annular jet method, is an encapsulation technique which allows to  
154 obtain core-shell capsules as shown in Fig.4A. Alginate solution and solutions containing the  
155 substances to encapsulate are simultaneously injected through a coaxial nozzle, which permits  
156 to obtain an annular jet formed by two concentric jets. The outer material represents the shell  
157 material, *i.e.* alginate solution, and the inner contains the solution with the target substance.  
158 The jet is then separated into droplets and collected in a gelling bath. As for the simple  
159 dripping process, different separative methods are possible: vibration, electrostatic forces,  
160 spinning disk or simple dropping into crosslinker solution (Oxley, 2012a).

161 In addition to the previously mentioned factors that impact beads size, a couple of factors are  
162 specific to coextrusion process to reach a given shape of core-shell capsules. For instance,  
163 nozzles sizes (internal/external) affect capsules size. Slow flow rate tends to produce larger  
164 capsules (drop by drop system) than capsules produced at high flow rate (Lucía et al., 2017;  
165 Oxley, 2012a).



166

167 **Fig. 4.** Scheme of a core-shell structural organization for capsules (A), scheme of a  
168 combination structural organization for capsules (B) and a photograph of a core-shell capsule.

169 Coextrusion technology allows production of capsules with a huge payload (over 90%) and a  
170 large range of capsules size can be obtained with a diameter from few microns to a several  
171 millimeters. The process is easily scaled-up for industrial use (Oxley, 2012a; Wang et al.,

172 2013). For example, Lucia et al (2017) worked on the production of almonds essential oil  
173 liquid-core capsules obtained by coextrusion process (Lucía et al., 2017). Silva et al (2018)  
174 studied the encapsulation of *Lactobacillus acidophilus* and compared the simple dripping  
175 method to the coextrusion technique. An additional protective function was observed for  
176 beads produced by coextrusion and especially for capsules obtained with a composite shell  
177 made of an alginate/shellac matrix (Silva et al., 2018).

### 178 3. Factors influencing alginate gelation and spherification

#### 179 3.1. Molecular weight of alginate

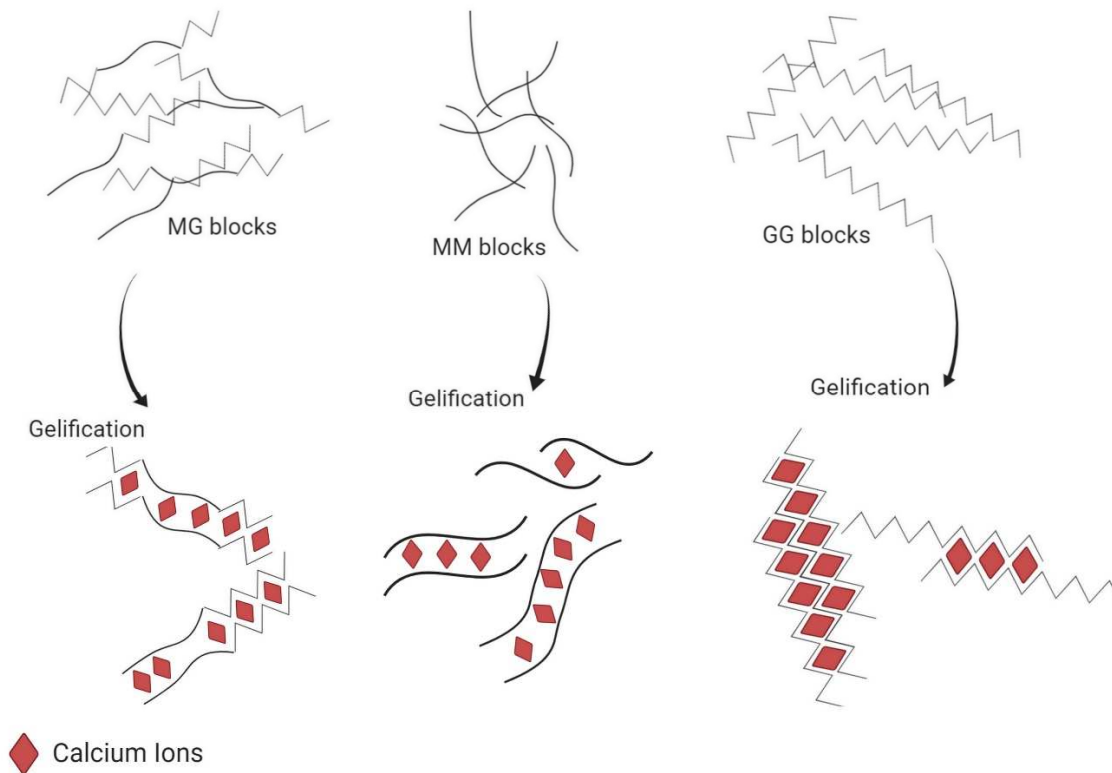
180 Generally, high molecular weight of alginate due to long polymeric chains results in a larger  
181 number of Ca-binding sites then tighter crosslinking in the gel (Hu et al., 2020). These  
182 interactions impact gelation process and produce a high viscosity gels, so that low molecular  
183 weight alginate weight alginate ( $1,43 \times 10^5 \text{ g mol}^{-1}$ ) viscosity is 2.4 times lower than higher  
184 molecular weight alginate ( $3,5 \times 10^5 \text{ g mol}^{-1}$ ) viscosity (0.05 Pa.s versus 0.12 Pa.s) in the same  
185 experimental conditions.

186 Gelation kinetics are also faster with increasing alginate molecular weight (Fernández Farrés  
187 & Norton, 2014). Moreover, similar conclusions have been found after acidic treatment of  
188 high molecular weight alginate so that low molecular weight alginate obtained produced low  
189 viscosity gels (Haug, Larsen, Smidsröd, et al., 1963; Jiao et al., 2019).

190 Effects of other novel processing methods have been studied. Such as high-pressure  
191 homogenization also called dynamic high pressure that caused a reduction of alginate  
192 molecular weight and viscosity under 300 MPa and after 5 cycles when a minimal viscosity  
193 plateau was observed (Harte & Venegas, 2009).

#### 194 3.2. M/ G ratio and distribution of G, M and GM units along the alginate chains

195 Alginate gelation is dependent of the percentage of G and M units, as a consequence of a  
196 higher degree of hindered rotation around the glycosidic linking. Because of this  
197 configuration, cross linking between alginate  $\text{Ca}^{2+}$  is mostly due to G units (Braccini et al.,  
198 1999; Feng et al., 2017; Jiao et al., 2019). M units moderate elasticity and lateral association  
199 in alginate gelation. Gels formed with high percentage of M units are reported as soft and  
200 elastic while those formed with high percentage of G units are described as compact and rigid  
201 (Leirvåg, 2017). Beads produced with low molecular weight and high G content alginate are  
202 the strongest, the biggest and best relevant to protect probiotics by encapsulation (Ramos et  
203 al., 2018).



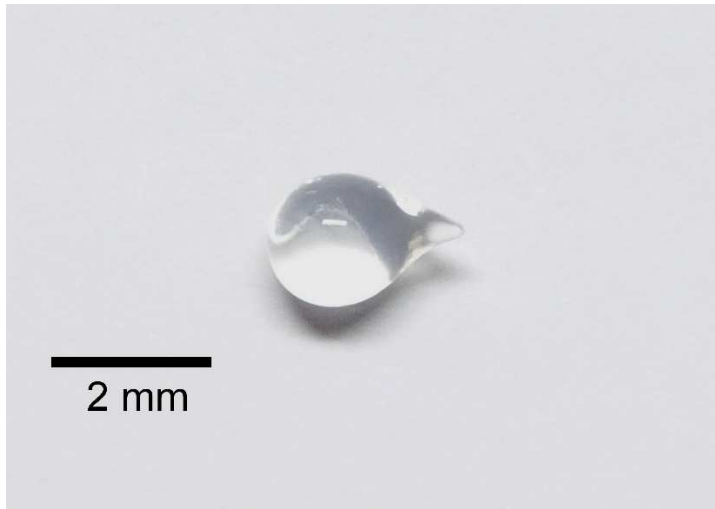
204

205 **Fig. 5.** Effect of calcium on the MG, MM and GG alginate units.

206 3.3. Alginate Concentration

207 Gelation can be impacted by alginate concentration. Fernández Farrés *et al.* (2013) found that  
 208 higher concentration of the biopolymer, in the range of 1% to 4% (w/w), induced higher  
 209 viscosity, elasticity rupture strength and gel hardness. As expected, higher concentration of  
 210 alginate molecules increased the number of cross-linking interactions with calcium ions and  
 211 reinforced gel structure (Fernández Farrés *et al.*, 2013). However, excessive concentration of  
 212 alginate reduced binding opportunities by decreasing  $\text{Ca}^{2+}$  diffusion through the matrix (J.  
 213 Yang *et al.*, 2020). Nevertheless, for moderate changes in alginate concentration from 1.3 to  
 214 1.7% [w/w], Hu *et al.* (2020) did not notice significant variations of alginate beads structure.

215 In the case of beads processing, alginate concentration has also an impact on beads shape in  
 216 alginate extrusion process, alginate beads tend to be more spherical when viscosity increases.  
 217 The result of a bead made with the appropriate viscosity can be found in Fig.3C.  
 218 Nevertheless, when viscosity is too high, beads are drop-shaped as shown in Fig.6 (Z. Q. Li *et al.*, 2013).  
 219



220

221 **Fig. 6.** Example of a drop-shaped alginate bead.

222 3.4. Concentration of calcium ions

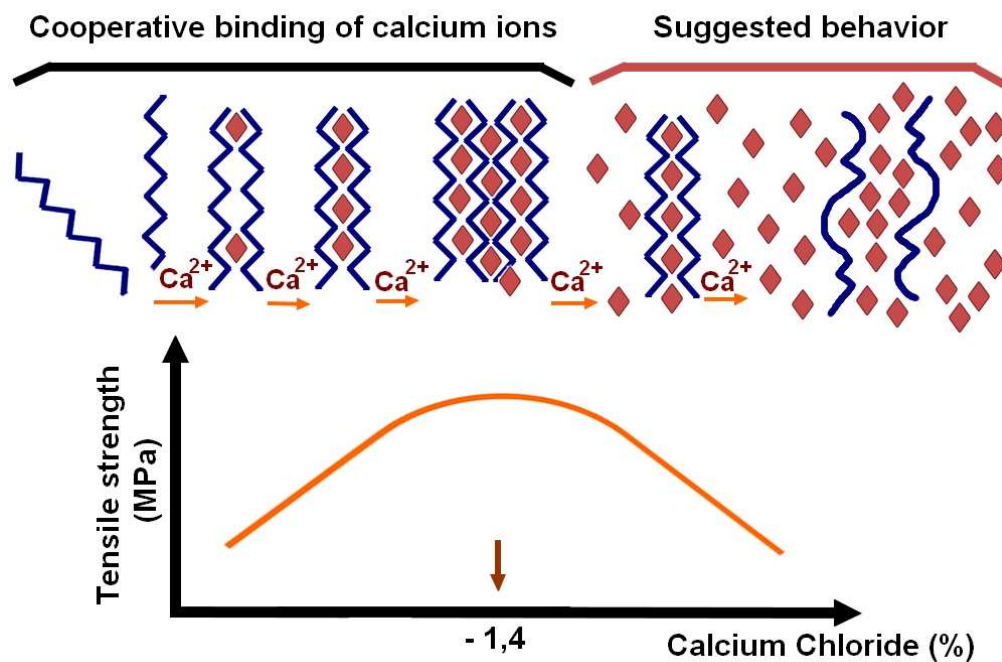
223 An increase of  $\text{Ca}^{2+}$  concentration results in an increase of cross-linking gelation rate. Faster  
224 diffusion of calcium ions in gels lead to higher gel viscosity, elasticity, and stability (J. Li et  
225 al., 2016). The calcium versus carboxyl group concentrations ratio is called the R value and  
226 describes effect of  $\text{Ca}^{2+}$  on alginate gelation and it is defined as follows:

227 
$$R = [\text{Ca}^{2+}]/[\text{COO}^-]$$

228 Alginate crosslinking with calcium ions can be described in three steps: (i) the mono-  
229 complexation at  $R < 0.25$ , (ii) dimerization at  $0.25 < R < 0.55$  resulting in formation of the egg-  
230 box model; and (iii) third step occurs at  $R > 0.55$  and results in the lateral association of the  
231 polymer chains forming intra of inter-clusters (Y. Fang et al., 2007).

232 Higher viscosity and maximum tensile stress of alginate gel are reached at around 1.4% (w/w)  
233 of  $[\text{CaCl}_2]$  for alginate concentration equaled to 127mM.  $\text{Ca}^{++}$  saturation of alginate chains  
234 can explain that maximum tensile stress occurred (Cuadros et al., 2012). At high calcium  
235 concentration calcium ions induce excessive positive charges that will segregate alginate  
236 chains leading to the gel disintegration. The mechanism between alginate structure and  
237 calcium chloride concentration is illustrated in Fig. 7.

238 It should be noted that, while producing alginate beads, a high calcium concentration also  
239 affects beads sphericity which tends to be less spherical at high calcium concentrations  
240 (Cuadros et al., 2012).



241  
 242 **Fig. 7.** Scheme representing the mechanism of strengthening and weakening of the alginate  
 243 gel as a function of the concentration of calcium chloride (Adapted from Cuadros et al., 2012)

### 244 3.5. Residence Time

245 The time during which alginate is into contact with calcium ions in gelation bath is called  
 246 residence time. When alginate drops are exposed to calcium, ions migrate from surface to  
 247 core. Required residence time for reticulation ( $t_{gel}$ ) can be calculated using Fick's second law  
 248 which can be expressed as:

$$249 \quad \frac{C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}$$

250 Where C is alginate concentration ( $mol/m^3$ ), t is time (s), D is diffusion substance coefficient  
 251 ( $m^2/s$ ) and x is the position (m).

252 Fick's second law can be approximated as:

$$253 \quad \frac{C}{t_{gel}} \approx D \frac{C}{L^2}$$

254 Where C represents alginate concentration ( $mol/m^3$ ), L is a characteristic length given by the  
 255 bead radius (m), and D the diffusion coefficient of calcium chloride in water:  $10^{-9}m^2/s$   
 256 (Evageliou et al., 2000). It has been demonstrated that after a residence time of 30 min,

257 samples exposed to different calcium concentrations showed similar behaviors (Cuadros *et al.*  
258 (2012). Residence time seems to have no effect on beads sphericity (Hartmann *et al.*, 2006).

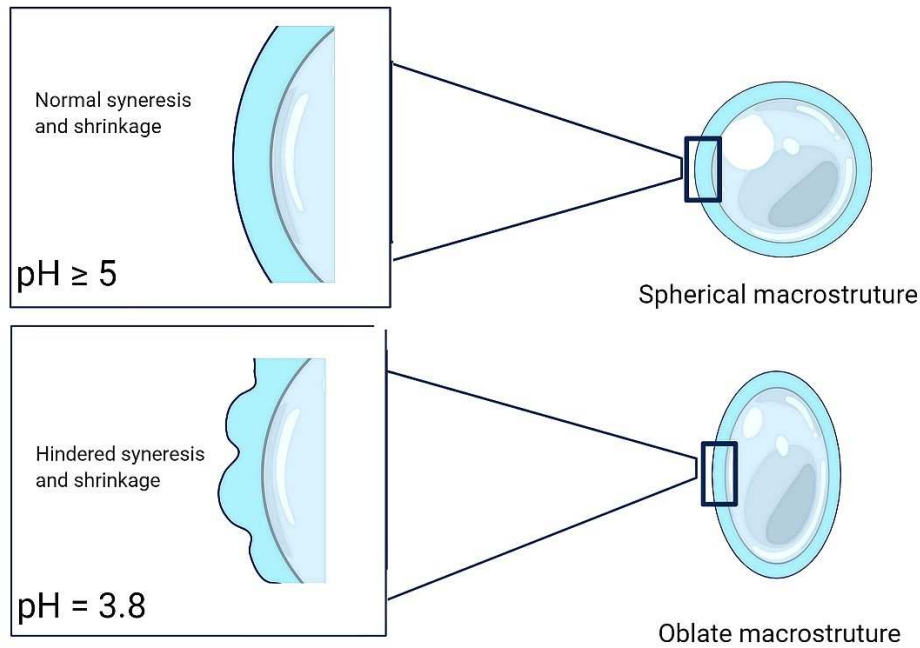
### 259 3.6. pH

260 pH impacts alginate water solubility and ion-binding as it determines the electrostatic charges  
261 of G and M units. M and G units have pKa of 3.38 and 3.65, respectively. Alginate polymer  
262 residues have pKa value usually estimated around 3.4 (Park *et al.*, 2019). Alginate composed  
263 of a high content of MG-sequences are more soluble at low pH and precipitate at pH around  
264 2.6 and 2.7 while high G units content alginate precipitate at pH around 3.25 (Haug, Larsen,  
265 Samuelsson, *et al.*, 1963; Sonogo *et al.*, 2016; Zazzali *et al.*, 2019). At higher pH values  
266 (below pH = 10.5) glycosidic bonds of alginate gel start to weaken and break leading to  
267 structural degradation (Zazzali *et al.*, 2019).

268 The microstructure of the alginate gel has been largely studied and it has been established that  
269 gel obtained at pH = 3.8 has a denser and interconnected microstructure due to stronger chains  
270 interactions. This phenomenon leads to less syneresis and shrinkage of the gel. Gels produced  
271  $\text{pH} \geq 5$  present lower interconnectivity in their microstructure and lower density, then  
272 allowing syneresis and shrinkage (Augst *et al.*, 2006; Jeong *et al.*, 2020; Moe *et al.*, 1992;  
273 Topuz *et al.*, 2012).

274 During preparation of alginate beads, interfacial tension is impacted by pH, lower pH values  
275 result in lower interfacial tension. pH also influences beads shape, beads produced in low pH  
276 gelation bath tend to be oblate, particularly in low alginate and ions concentrations, while  
277 those produced at  $\text{pH} > 4$  tend to be spherical (Chuang *et al.*, 2017; Sonogo *et al.*, 2016;  
278 Zazzali *et al.*, 2019).

279



280

281 **Fig. 8.** Macrostructure of alginate beads synthesized at pH= 3.8 and pH ≥ 5.

282 3.7. Temperature

283 Gelation temperature has an impact on alginate gel microstructure. High temperature seems to  
 284 enhance and facilitate calcium ions penetration through alginate network while low  
 285 temperature slows down diffusion and gelation process. Low temperature led to more ordered  
 286 and regular network and higher temperature produced a denser network with lower young's  
 287 modulus (Jeong et al., 2020). At physiological gelation temperature around 36-40°C, an  
 288 alginate gel with high young's modulus was produced (Leo et al., 1990; Oates & Ledward,  
 289 1990).

290 Jeong et al. (2020) reported that an increase in gelation temperature led to lower alginate  
 291 viscosity and reduced beads size. Gelation temperature seems also to influence beads  
 292 sphericity that increased with higher gelation temperature. Besides, a high beads rupture  
 293 strength was reported at low gelation temperature (5°C) (Cerciello et al., 2017; Y. Gu et al.,  
 294 2018; Sanchez-Ballester et al., 2019; Vu et al., 2017). If the gels are stable at range 0 to  
 295 100°C, more G units content increase alginate gels thermal stability (Hartmann et al., 2006).  
 296 Above 100°C the structure began to depolymerize and at 180°C the gel was totally  
 297 destructured (Aida et al., 2010).

298 3.8. Ionic strength

299 Gelation is impacted by solution ionic strength. Low ionic strength allows gel formation, but  
 300 high level of monovalent salts competed with calcium ions for carboxyl alginate groups  
 301 resulting in an instable and weak system. Van Leusden et al. (2017) reported a structural  
 302 alteration of alginate beads exposed to high NaCl concentration. This modification might be  
 303 due to a decrease of electrostatic attractive interactions into the system and/or increase in  
 304 beads pore size resulting in the release of the encapsulated compounds (Jiao et al., 2019).  
 305 Apparently, salts ions dislocated some Ca<sup>2+</sup> leading to gel disintegration. The extent of  
 306 network destructure increased with higher salts concentration (Jiao et al., 2019).

307 3.9. Shear rate

308 Jiao et al. (2019) reported the influence of shear rate on the gelation method described as “*in*  
 309 *situ* addition of calcium”, to favor homogenous gelation by avoiding disadvantages of  
 310 external gelation. Shear rate had an impact on gelation kinetics: when shear was applied at the  
 311 beginning of gelation process, the onset time of gelation was higher and gel particles were  
 312 faster formed (Hernández-González et al., 2020; Jiao et al., 2019; Puguán et al., 2014).  
 313 However, gel viscosity decreased with increasing shear rate applied demonstrating the  
 314 relation between shear rate and gel rheology (Mancini & McHugh, 2000).

315  
 316 In conclusion for factors influencing alginate gelation and spherification, Table 2 summarizes  
 317 the effect of the principal parameters on alginate beads gelation. We choose to present the  
 318 clearer effect for an efficient production of alginate bead with desired properties. Some  
 319 parameters have not been enough studied to appear in our summarized Table 2 and factors as  
 320 pH has an effect depending on application.

321 **Table 2**  
 322 Effects of increased factors influencing alginate gelation and spherification.

Enhanced Parameters	Effects of gel formation				Effects on beads formation			
	Viscosity	Compactness	Network stability	Ca <sup>2+</sup> penetration kinetics	size	strongness	sphericity	rupture strength
	Alginate structure							
↗Molecular weight	↗	↗	↗	↘	↘	↗	↗↘	↗
↗M/G ratio	↘	↘	↘	-	-	↘	-	↗
	Experimental environment							
↗ [alginate]	↗	↗	↗	↘		↗	↗↘	
↗ [Ca <sup>2+</sup> ]	↗	↗	-	↗	-	-	↘	
↗Temperature	Optimum at 36-40°C	↘	↘	↗	↘	-	↗	↘



## 323 4. Composite Alginate gels

324 Composite gels with alginate and other polymers have been studied for multiple purposes and  
325 in many fields, such as encapsulation, packaging films, or edible films. This interest results  
326 from the ambition to modify gel mechanical properties (Poncelet et al., 1992), brings new  
327 characteristics and extends its use. Polysaccharides and proteins are the main components  
328 used for copolymerization. Depending on copolymer nature mixed with alginate, gelation  
329 process varies as described below.

### 330 4.1. Polysaccharides as co-polymer

#### 331 4.1.1. Pectin

332 Pectin is a biopolymer extracted from plant cell walls like citrus peel or apple pomace. B.  
333 Zhang et al. (2019) reported that pectin gelation is enhanced by addition of guluronic units.  
334 Encapsulation in alginate-pectin gel exhibited a significant reduction of anthocyanins  
335 degradation (Guo et al., 2018; B. Zhang et al., 2019). A lower energy consumption has also  
336 been demonstrated in jelly candy manufacturing using alginate-pectin cold gelation instead of  
337 traditional heating process (de Avelar & Efrain, 2020). In the medical field, alginate-pectin  
338 hydrogel presented great improvement of mechanical properties, water absorption, thermal  
339 stability, and drug release for wound dressing application (Oh et al., 2020; Rezvanain et al.,  
340 2017). In food industry, pectin-alginate-gelatin composite gels showed promising results for  
341 probiotics encapsulation, by increasing stability of encapsulated bacteria (Vaziri et al., 2018).

#### 342 4.1.2. Gums

##### 343 4.1.2.1. Guar gum

344 Guar gum is extracted from guar beans (*Cyamopsis tetragonoloba*). It is composed of a linear  
345 chain of (1-4)- $\beta$ -D-mannopyranose linked with  $\beta$ -galactopyranosyl groups. Guar gum is water  
346 soluble and usually used in coating. Guar gum is used as thickener of alginate-guar gum gel  
347 (Mousavi et al., 2019; Rastegar & Atrash, 2020).

348 Gel made with alginate/guar gum was stronger at lower calcium level than pure alginate gel  
349 and presented a high elasticity and rigidity due to a lower degree of porosity. It reduced  
350 syneresis and shrinkage during storage. Guar gum addition to alginate enhanced network  
351 stability in acidic conditions and thermal processing (Mousavi et al., 2019, 2020). Alginate-  
352 guar gum gel exhibited an improved polyphenols and betacyanins encapsulation (Aguirre  
353 Calvo et al., 2019), and enhanced preservation of antioxidant activity for encapsulated fatty  
354 acids (Aguirre-Calvo et al., 2020).

355 4.1.2.2. Gum arabic

356 Also known as acacia gum, gum arabic is a natural polymer extracted from acacia trees and it  
357 consists of  $\beta$ -D-galactopyranosyl units branched with side chains of L-rhamnose, D-galactose,  
358 L-arabinose and D-glucuronic acid. This polymer is largely used in food, cosmetic and  
359 pharmaceutical industries as thickening agent and stabilizer (Tsai et al., 2017). Interaction  
360 between alginate and gum arabic has been recently studied by Sabet et al (2021). These  
361 authors reported interactions between polymers at pH 7 and a stronger synergy at pH 4, due to  
362 ionic interactions between respective negative and positive charges of alginate and gum  
363 arabic. The ionic binding between alginate and gum arabic results in an increase of gel  
364 viscosity and viscoelasticity (Sabet et al., 2021).

365 The alginate/gum arabic composite exhibited great results in drug encapsulation. Moreover,  
366 Nayak et al (2012) reported an encapsulation efficiency of  $86.02 \pm 2.97\%$ .

367 4.1.2.3. Other gums

368 Other gums such as galbanum gum or basil seed gum have been investigated recently for  
369 different applications, especially in food packaging field. Addition of gum to alginate matrix  
370 brings a real advantage in terms of barrier function against gas, water, oil transport (Hamedi et  
371 al., 2017; Mousavi et al., 2020).

372 4.1.3. Chitosan

373 Chitosan is a linear polysaccharide extracted from crustaceans' shells chitin. It is a nontoxic  
374 biopolymer formed by D-glucosamine and N-acetyl glucosamine units. Chitosan is used in  
375 numerous field due to its numerous properties, *i.e.* antioxidant, lipid binding, anti-  
376 inflammatory and antimicrobial effects (Kulig et al., 2016).

377 Chitosan gel viscosity increases with pH. The strongest alginate-chitosan gels were obtained  
378 with increasing pH and chitosan weight percentage, but considerable viscoelasticity and  
379 maximum encapsulation capability were obtained at neutral pH (Afzal et al., 2018).

380 Combining alginate and chitosan improves mechanical and chemical gel properties and favors  
381 thermal resistance. Chitosan has been largely investigated in coating alginate capsules, due to  
382 its positive charges that lead to an ionic complexation with negative charges of alginate. This  
383 complexation allows chitosan solubilization into a non-acetic acid or hydrochloric solutions.  
384 Thereby, coating with chitosan enhanced capsules membrane thickness and their protective  
385 abilities (Fernandes et al., 2020; Raeisi et al., 2020; Taqieddin & Amiji, 2004).

386 Composites exhibited great results for globulin preservation during thermal treatment (Kulig  
387 et al., 2016; Romo et al., 2020). De Prisco et al (2015) reported a better stress tolerance to  
388 food processing for probiotics encapsulated with alginate-chitosan composites.  
389 Alginate/chitosan composites have been examined for oil co-encapsulation or  
390 microencapsulation to protect oil from oxidation, capsules showed good oxidative stability  
391 and oxidation products were lower for chitosan/alginate compared to non-encapsulated oil.  
392 Results also showed an effective oil vehicle for nutraceuticals (Atencio et al., 2020; Hamed et  
393 al., 2020). Microwave treatments have also been considered for drugs controlled-release  
394 alginate/chitosan microbeads. Depending on experimental conditions, microwave treatments  
395 could induce cross-linkage or breakdown. Thus, Wong et al. (2002) reported a retarded drugs  
396 release from alginate/chitosan beads treated at 80 W for 10 minutes as a consequence of  
397 enhanced alginate/chitosan cross-linking.

#### 398 4.1.4. Carrageenan

399 Carrageenan is a sulphated polygalactan that can be obtain from red seaweeds, it contains 15  
400 to 54% charged sulphate ester groups. This polysaccharide is formed by D-galactose and 3,6-  
401 anhydro-galactose linked by  $\alpha$ -1,3 and  $\beta$ -1, 4-glycosidic bonds. There are various  
402 carrageenan types:  $\lambda$ ,  $\kappa$ ,  $\iota$ ,  $\epsilon$ ,  $\mu$ . The classification depends on the sulphate ester groups number  
403 and position (Necas & Bartosikova, 2013). Alginate/carrageen composite have been largely  
404 investigated in edible films formulation for food and non-food applications; good barrier and  
405 protection properties have been reported (Tavassoli-Kafrani et al., 2016).  
406 Combination of  $\kappa$ -carrageenan and alginate enhances gel rigidity and mechanical stability.  
407 Carrageenan/alginate composite gel appeared more transparent than conventional alginate gel  
408 with a compact and smooth surface. Addition of carrageenan tends also to improve thermal  
409 properties, elastic modulus, gel water content, and swelling which is induced by sulphate  
410 groups from carrageenan molecules. Beads formed with carrageenan/alginate composite had a  
411 larger diameter than alginate beads (L. Li et al., 2019; Mohamadnia et al., 2008).  
412 The pH-dependent properties allowed use of carrageenan/alginate complex in a pH-  
413 responsive beads for insulin encapsulation. In this study, protection against acidic condition  
414 and controlled release were investigated (Lim et al., 2017). Good protection was observed at  
415 pH=1.2 and progressive release with good kinetics was noticed at pH=7.4. This point made  
416 composite carrageenan/alginate beads promising encapsulation matrix for oral insulin intake.  
417 L.Gu et al (2021) worked on immunoglobulin encapsulation for controlled release and

418 reported high encapsulation efficiency. Composite exhibited a denser internal microstructure  
419 and a good protection in acidic gastric conditions (L. Gu et al., 2021).

#### 420 4.1.5. Starch

421 One of the most abundant and cheap natural polysaccharides is starch. Starch contains  
422 amylose and amylopectin with linkage of  $\alpha$ -D-glucopyranose molecules. Starch is largely  
423 used in hydrogel production, due to its free hydroxyl groups. Starch works in synergy with  
424 alginate. Water absorption capacity and encapsulation efficiency are improved. For instance,  
425 López Córdoba et al. (2013) reported improvement of encapsulation efficiency from 55 to  
426 65% of liquid extract of yerba mate (a high polyphenols tea-like beverage) (da Silva  
427 Fernandes et al., 2019; López Córdoba et al., 2013). The synergy between starch and alginate  
428 depends on chemical structures of both polymers, but also on processing conditions, such as  
429 pH, ionic strength and salts concentration (da Silva Fernandes et al., 2019). Gelation of  
430 alginate/starch composite occurs using different divalent cations ( $Mn^{2+}$ ,  $Zn^{2+}$ ,  $Ca^{2+}$  for  
431 example), and characteristics of the composite gel vary from an ionic crosslinker to another  
432 (da Silva Fernandes et al., 2019).

433 Addition of starch to alginate creates composite gels with variable porosity depending on  
434 starch type, but starch addition provides a slower release of encapsulated substances (Feltre,  
435 Almeida, et al., 2020; Okunlola & Adewusi, 2019). Starch type impacts gel water retention,  
436 stability, and digestibility. Those features must be considered in the formulation of controlled  
437 release beads. For instance, Hassan et al (2020) obtained promising results for gradual release  
438 of nisin encapsulation (Feltre, Almeida, et al., 2020; Hassan et al., 2020).

439 Feltre, Sartori, et al (2019) recently reported an efficient encapsulation of oil with starch  
440 incorporation in alginate systems, due to greater oil dispersion into oil/starch/alginate  
441 emulsion. Beads formed by oil/starch/alginate emulsions containing equal proportions of  
442 alginate and starch presented good results in terms of antioxidant activity preservation and  
443 storage stability (Feltre, Sartori, et al., 2020). Similar results concerning encapsulated  
444 component stability have been obtained by Jain et al (2020) for lycopene encapsulation with  
445 an enhanced bioaccessibility. This could be promising for nutraceuticals and functional foods  
446 application (Jain et al., 2020). As an example, alginate/starch/gelatin composite has been  
447 recently investigated for encapsulation of  $\beta$ -carotene in oil/starch/alginate/gelatin emulsion.  
448 The study demonstrated a great stability and oxidation reduction for encapsulated  $\beta$ -carotene  
449 compared to  $\beta$ -carotene in free oil. Those results are very important to extend shelf-life of

450 components sensitive to oxidation during processing for food or drugs application (Guedes  
451 Silva et al., 2021).

#### 452 4.2. Proteins/ polypeptides as co-polymer

##### 453 4.2.1. Whey Proteins

454 Under specific conditions, proteins can be used as cations source for alginate gel cross-  
455 linking. Thus, alginate/proteins composite hydrogels are largely investigated and exhibited  
456 great results in protein carriers with controlled release kinetics (Rahmani & Sheardown,  
457 2018). Denatured whey protein isolates and alginate composite gels have shown great  
458 thermal stability after 1 minute at 63 and 72°C and a good resistance to acidic conditions for  
459 encapsulated probiotics by extrusion or spray-drying process (H. Liu et al., 2018;  
460 Praepanitchai et al., 2019; Rajam et al., 2012). Dehkordi et al (2020) worked on an  
461 alginate/whey protein isolate composite for probiotics encapsulation and reported a highest  
462 survivability of *lactobacillus acidophilus* for an optimal matrix composed of 4.54% (w/v) of  
463 alginate and 10% (w/v) of whey protein isolate (Dehkordi et al., 2020).

464 Recent studies showcased interest of protein addition in edible alginate films. Chkravartula et  
465 al (2019) reported significant beneficial effects of whey protein on the physico-chemical,  
466 rheological, mechanical and optical properties of edible packaging. Addition of whey proteins  
467 to alginate decreased film viscosity and enhanced its hydrophobicity and reduced film water  
468 absorption (Chakravartula et al., 2019).

##### 469 4.2.2. Gelatin

470 Gelatin is largely studied in coacervation process, which is a spontaneous phase separation  
471 between an insoluble complex and of another aqueous phase that occurs with pH change (de  
472 Matos et al., 2018). Consequently, gelatin and alginate are pertinent materials for  
473 encapsulation process by coacervation. Promising results have been obtained for essential oils  
474 microencapsulation, especially with a 4% gelatin and high essential oils concentration matrix,  
475 with a 73.7% encapsulation efficiency, and a good terpenes preservation (de Matos et al.,  
476 2018; Heckert Bastos et al., 2020). Moreover, Y. Li et al (2019) reported a good effectiveness  
477 of alginate/arabic gum /gelatin composite for preservation of encapsulated antioxidant  
478 substances for oral delivery (Y. Li et al., 2019). Lemos et al (2017) also observed high  
479 encapsulation efficiency in the production of buriti oil microcapsules through coacervation  
480 (Lemos et al., 2017).

481 M. Lopes et al (2017) worked on probiotics encapsulation using an alginate/gelatin composite  
482 matrix. Spherical and regular beads were obtained, and higher cells viability was reported at

483 1% w/v alginate and 0.1% w/v gelatin. Therefore, beads contained up to  $10^5$  CFU  $\text{g}^{-1}$  after 4  
484 months of storage (M. Lopes et al., 2017).

485 **Table 3**

Co-polymers	Composition	Positive effect of co-polymer addition to alginate	Application	Refs
Pectin	$\alpha$ -D-galacturonic acid $\alpha$ -L-rhamnose	Encapsulated substance stability. Gel water absorption and thermal stability. Lower gel viscosity.	Probiotics encapsulation. Cold gelation for candy manufacturing.	Belscak-Cvitanovic et al., 2015; de Avelar & Efraim, 2020; Oh et al., 2020; Vaziri et al., 2018
Guar gum	$\beta$ -D-mannopyranose $\beta$ -galactopyranosyl	Strong gel Low porosity gel Network stability to heat and acidic conditions	Coating for food packaging Fatty acids encapsulation	Aguirre-Calvo et al., 2020; Mousavi et al., 2019; Rastegar & Atrash, 2020
Gum arabic	$\beta$ -D-galactopyranosyl L-rhamnose D-galactose	High viscosity and viscoelasticity gel Encapsulation efficiency	Drugs encapsulation Radish juice liquid-core encapsulation	Nayak et al., 2012; Sabet et al., 2021; Tsai et al., 2017
Chitosan	D-glucosamine N-acetyl-glucosamine	Mechanical, chemical properties and thermal stability. Gel protective abilities and permeability	Protein encapsulation Omega-3 rich oil encapsulation Coating capsules	Fernandes et al., 2020; Hamed et al., 2020; Kulig et al., 2016; Romo et al., 2020
Carrageenan	D-galactose 3,6-anhydro-galactose	Gel rigidity and mechanical stability (elastic modulus). Water content, thermal properties, and swelling.	Immunoglobulins encapsulation Edible films for food application	L. Gu et al., 2021; L. Li et al., 2019; Necas & Bartosikova, 2013; Tavassoli-Kafrani et al., 2016
Starch	Amylose Amylopectin $\alpha$ -D-glucopyranose	Water absorption. Better oxidation stability Capsule digestibility	Spirulina encapsulation Edible films for food packaging Nisin or lycopene encapsulation	Feltre, Sartori, et al., 2020; Jain et al., 2020; Rajmohan & Bellmer, 2019; Wu et al., 2001
Whey protein	$\alpha$ -lactalbumin $\beta$ -lactoglobulin Albumin Immunoglobulins	Gel antioxidant properties Active protection against visible light, heat, and acidic conditions	Films for food packaging Probiotics encapsulation	Chakravartula et al., 2019; Dehkordi et al., 2020; Oliveira Filho et al., 2019; Praepanitchai et al., 2019
Gelatin	Amino acids	Encapsulation efficiency Set up coacervation process	Essential oils encapsulation Probiotics encapsulation Antioxidant encapsulation	Heckert Bastos et al., 2020; Y. Li et al., 2019; S. Lopes et al., 2017

486 Co-polymers for alginate composite gels for encapsulation.

487

488 5. Other Encapsulation processes using alginate

489 5.1. Spray-drying

490 Spray-drying is known since the 1930's as one of the earliest encapsulation techniques due to  
491 its simplicity of application and high productivity among many other advantages (Table 4) (Z.  
492 Fang & Bhandari, 2012). The process principle is injection of a solution (emulsion or  
493 dispersion with active compounds) through a simple nozzle or centrifugal wheel. The liquid is  
494 then atomized into small droplets which fall and meet hot air in a drying chamber. After water  
495 evaporation, particles fall to the bottom of the chamber and are collected in a powder form.

496 Spray-drying process is applied in many fields and several wall materials can be used.  
497 Alginates exhibit good results for volatiles and emulsions encapsulation (Z. Fang & Bhandari,  
498 2012; Rodríguez et al., 2016).

499 The efficiency of spray-drying process depends strongly on inlet air temperature, particularly  
500 for thermolabile substance encapsulation, and can affect particles chemical and physical  
501 properties in addition to core materials stability (X. Sun et al., 2020). In this perspective, X.  
502 Sun et al (2020) studied the impact of inlet air temperature on spray-drying  
503 microencapsulation of lipophilic carvacrol in an oil-in-water emulsion with alginate/pectin  
504 composite shell material. They reported an optimum temperature for spray-drying  
505 encapsulation between 100 and 130°C for carvacrol. However, even if a higher temperature  
506 tends to increase encapsulation efficiency, it leads to reduce moisture content and retention  
507 efficiency (X. Sun et al., 2020).

508 Recently, Stobel et al. (2018) presented a new encapsulation process using spray-drying  
509 technology and called the Cross-Linked Alginate Microcapsules process (CLAMs process). It  
510 uses a calcium ions source which is insoluble at feed pH, but when solution is atomized the  
511 base present in the matrix evaporates which drop its pH and allows calcium ions to solubilize  
512 and react with alginate. The advantage of this process is to reduce the multi-step conventional  
513 spray-drying process into a one-step operation (Jeoh et al., 2020; Strobel et al., 2018).

514 Stobel et al (2020) worked on fish oil encapsulation using the CLAMs process and reported  
515 good results in term of shelf-life of encapsulated fish oil in a shell material made of modified  
516 starch and alginate. Some promising enteric release properties were also observed for  
517 gastrointestinal delivery of lipophilic bioactives (Strobel et al., 2020).



518        5.2.    Spray-cooling

519    Also known as spray-congealing or prilling, spray-cooling is a process that allows atomization  
520    of a liquid into small particles. The process is similar to spray-drying, the solution is injected  
521    through a pressure nozzle, vibrating nozzle or a spinning disk into an atomization chamber.  
522    Unlike spray-drying, the atomized liquid is cooled and hardened (Oxley, 2012b).

523    Encapsulation by spray-cooling allows encapsulation of actives ingredients dispersed into a  
524    liquid matrix. Instead of spray-drying, encapsulation by spray-cooling allows encapsulation of  
525    thermolabile substances (Oxley, 2012b). This process is used in food and nutraceutical  
526    industry for preservation of flavor (Oxley, 2012b), vitamins (Chalella Mazzocato et al., 2019),  
527    or other bioactive ingredients like probiotics (Dong et al., 2013).

528        5.3.    Coacervation

529    Coacervation occurs in a colloidal system and leads to liquid/liquid phase separation between  
530    a colloid-rich phase also known as coacervate phase and a solvent-rich diluted phase. The  
531    coacervate phase can be composed of a single polyelectrolyte. In this case, it is called simple  
532    coacervation. Complex coacervation occurs when the coacervate phase is composed of an  
533    ionic complex of two opposite charges polyelectrolytes as for alginate complex coacervation  
534    for encapsulation purposes. Solution pH strongly impacts coacervate formation, in addition to  
535    polyelectrolytes concentrations and their mixing ratio, ionic strength and temperature (Gorji et  
536    al., 2018; Thies, 2012).

537    Combination of proteins and alginate is often studied for delivery systems, and in many fields  
538    such as food, cosmetic and pharmaceutical industries. Gorji et al (2018) worked on  
539    characterization of alginate, beta-lactoglobulin and bovine serum albumin interactions in  
540    solution. A protein-alginate ratio of 2:1 was used for this study. Being amphoteric proteins,  
541    Beta-lactoglobulin and bovine serum albumin have an isoelectric point of 5.2 and 5,  
542    respectively. They present positive charges at lower pH where alginate exhibit negative  
543    charges at low pH. Therefore, complex coacervation occurred mainly at pH range between  
544    ~1.6 and 4.2 where due to opposite charges of alginate and proteins (Gorji et al., 2018).

545    Another complex coacervation using gelatin and alginate has also been largely studied for  
546    encapsulation. Alginate, negatively charged at low pH, can form a complex coacervate with  
547    gelatin for essential oil encapsulation. Heckert Bastos et al. (2020) reported an optimum ratio  
548    of 6:1 gelatin to sodium alginate and a pH of 4 for black pepper essential oil encapsulation  
549    with an efficiency up to 82%. Similar efficiency result (74%) has been reported for citronella

550 essential oil encapsulation using 4% of gelatin and 10% of alginate. Microcapsules with a 434  
551  $\mu\text{m}$  diameter have been obtained with promising results for controlled release (de Matos et al.,  
552 2018).

553 For olive oil encapsulation, microcapsules sizes increase with the increase of oil amount but  
554 decreases with the increase of surfactant amount. Besides, FTIR spectra of free olive oil and  
555 microencapsulated oil have shown same characteristics so that no interactions between oil and  
556 alginate/gelatin composite have been exhibited (Devi et al., 2012).

557

**Table 4**

558

Advantages and limitations of principal encapsulation methods using alginate.

<b>Methods</b>	<b>Advantages</b>	<b>Limitations</b>	<b>Refs</b>
Extrusion	<ul style="list-style-type: none"> <li>- Easy application and industrialization</li> <li>- Hydrophilic matrix</li> <li>- Encapsulation of a large variety of substances</li> <li>- Protection against oxidation</li> <li>- Long shelf-life</li> <li>- various capsules size</li> </ul>	<ul style="list-style-type: none"> <li>- Low payload</li> <li>- Requirement of thermal treatment not compatible with heat-sensitive ingredients encapsulated</li> <li>-High porosity of microcapsules</li> <li>-Pure oil encapsulation in complicated</li> </ul>	Ching et al., 2017; Gouin, 2004.
Coextrusion	<ul style="list-style-type: none"> <li>-Large payload</li> <li>-Slow-release kinetics compared to other methods</li> <li>-Large variety of core materials</li> <li>-Scaled up process</li> <li>-Additional protective barrier brought by the shell</li> <li>- Encapsulated material is surrounded by wall material</li> </ul>	<ul style="list-style-type: none"> <li>- Risk of nozzles obstruction due to their small size</li> <li>- Process highly-dependent on core and shell fluid rates</li> <li>- Multifluidic technology expensive costs vs other methods</li> <li>-Difficulty to encapsulate extremely viscous matrix</li> </ul>	Gouin, 2004; Oxley, 2012a; Silva et al., 2018.
Spray-drying	<ul style="list-style-type: none"> <li>-Inexpensive technology and availability of the equipment</li> <li>- Possibility to perform double or triple encapsulation</li> <li>- Good encapsulation efficiency</li> <li>- Good stability for storage</li> <li>-Quick process.</li> <li>-Low product volume for reduced transport cost</li> </ul>	<ul style="list-style-type: none"> <li>- Limited shell materials choice</li> <li>- Exposition risk due to volatiles compounds at high temperature</li> <li>- Oxidation and degradation of thermolabile substances</li> <li>- Particles agglomeration</li> <li>- Particules size control</li> </ul>	Fang and Bhandari, 2012; Rodríguez et al., 2016; X. Sun et al.,2020.
Spray-cooling	<ul style="list-style-type: none"> <li>-Inexpensive technology for encapsulation</li> <li>-Large possibility of materials</li> <li>-Fast release (depending on the main objective)</li> <li>-Controlled size and higher specific surface area vs particles spray-dried.</li> </ul>	<ul style="list-style-type: none"> <li>- Weak protection on particles surfaces</li> <li>- High energy and cost required</li> <li>-Longer process time vs spray-drying</li> <li>- Handling and storage specific requirements</li> </ul>	Gouin, 2004; Liliana and Vladimir, 2013; Oxley, 2012b.
Complex coacervation	<ul style="list-style-type: none"> <li>-High payload</li> <li>-Controlled release possibilities</li> <li>-Flexible regarding shell material</li> <li>-Large particles size range</li> <li>-No specific equipment required</li> </ul>	<ul style="list-style-type: none"> <li>-Expensive process</li> <li>-Technique complexity</li> <li>- Low size range of capsules</li> <li>- Cohesion and adhesion of microcapsules.</li> <li>- Presence of solvents residues in spheres</li> </ul>	Bouquerand et al., 2012; Gouin, 2004; Ozkan et al., 2019; Saravanan and Rao, 2010;

559        6. Conclusion

560 Alginates are widely used and investigated polymers for a large variety of technical  
561 applications as thickeners or stabilizers but also in spherification processes for their gelation ability  
562 to encapsulate sensitive materials. They are Generally Recognized as Safe (GRAS), being nontoxic,  
563 nonantigenic, satisfactorily biocompatible, and sufficiently biodegradable.

564 Alginates gelation depends on polymer chemical structure counting its molecular weight and  
565 G/M units ratio and distribution along the alginate chain. Experimental environment greatly  
566 influences alginate gelation including pH, temperature, polymer concentration, crosslinker  
567 concentration, residence time and ionic strength.

568 Alginates have the advantage to be an encapsulation material adapted for most encapsulation  
569 processes especially spherification (extrusion and coextrusion) through their biocompatibility and  
570 capability to form a hydrogel through ionic cross-links with divalent cations, such as calcium  
571 ions. However, alginates have some limitations in certain applications. Therefore, alginates can  
572 work in synergy with many polymers (e.g. polysaccharides and proteins). Thus, composite  
573 alginate gels are widely studied recently in order to improve alginate encapsulation and  
574 spherification properties, including encapsulation efficiency and payload, storage stability and  
575 barrier properties.

576 Alginates are certainly one of the most promising encapsulation materials.

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- 586 1. Adli, S. A., Ali, F., Azmi, A. S., Anuar, H., Nasir, N. A. M., Hasham, R., & Idris, M. K. H. (2020). Development of  
587 biodegradable cosmetic patch using a polylactic acid/phycoerythrin-alginate composite. *Polymers*, 12(8), 1669.  
588 <https://doi.org/10.3390/POLYM12081669>
- 589 2. Afzal, S., Maswal, M., & Dar, A. A. (2018). Rheological behavior of pH responsive composite hydrogels of  
590 chitosan and alginate: Characterization and its use in encapsulation of citral. *Colloids and Surfaces B:  
591 Biointerfaces*, 169, 99–106. <https://doi.org/10.1016/j.colsurfb.2018.05.002>
- 592 3. Aguirre Calvo, T. R., Santagapita, P. R., & Perullini, M. (2019). Functional and structural effects of hydrocolloids  
593 on Ca(II)-alginate beads containing bioactive compounds extracted from beetroot. *Lwt*, 111(May), 520–526.  
594 <https://doi.org/10.1016/j.lwt.2019.05.047>
- 595 4. Aguirre-Calvo, T. R., Molino, S., Perullini, M., Rufián-Henares, J. Á., & Santagapita, P. R. (2020). Effect of in  
596 vitro digestion-fermentation of Ca(II)-alginate beads containing sugar and biopolymers over global antioxidant  
597 response and short chain fatty acids production. *Food Chemistry*, 333(July), 127483.  
598 <https://doi.org/10.1016/j.foodchem.2020.127483>
- 599 5. Agulhon, P., Robitzer, M., David, L., & Quignard, F. (2012). Structural regime identification in ionotropic alginate  
600 gels: Influence of the cation nature and alginate structure. *Biomacromolecules*, 13(1), 215–220.  
601 <https://doi.org/10.1021/bm201477g>
- 602 6. Aida, T. M., Yamagata, T., Watanabe, M., & Smith, R. L. (2010). Depolymerization of sodium alginate under  
603 hydrothermal conditions. *Carbohydrate Polymers*, 80(1), 296–302. <https://doi.org/10.1016/j.carbpol.2009.11.032>
- 604 7. Arriola, N. D. A., Chater, P. I., Wilcox, M., Lucini, L., Rocchetti, G., Dalmina, M., Pearson, J. P., & de Mello  
605 Castanho Amboni, R. D. (2019). Encapsulation of stevia rebaudiana Bertoni aqueous crude extracts by ionic  
606 gelation – Effects of alginate blends and gelling solutions on the polyphenolic profile. *Food Chemistry*, 275(August  
607 2018), 123–134. <https://doi.org/10.1016/j.foodchem.2018.09.086>
- 608 8. Atencio, S., Maestro, A., Santamaría, E., Gutiérrez, J. M., & González, C. (2020). Encapsulation of ginger oil in  
609 alginate-based shell materials. *Food Bioscience*, 37(July). <https://doi.org/10.1016/j.fbio.2020.100714>
- 610 9. Augst, A. D., Kong, H. J., & Mooney, D. J. (2006). Alginate hydrogels as biomaterials. *Macromolecular  
611 Bioscience*, 6(8), 623–633. <https://doi.org/10.1002/mabi.200600069>
- 612 10. Bajpai, M., Shukla, P., & Bajpai, S. K. (2016). Ca(II)+Ba(II) ions crosslinked alginate gels prepared by a novel  
613 diffusion through dialysis tube (DTDT) approach and preliminary BSA release study. *Polymer Degradation and  
614 Stability*, 134, 22–29. <https://doi.org/10.1016/j.polymdegradstab.2016.09.027>
- 615 11. Bannikova, A., Evteev, A., Pankin, K., Evdokimov, I., & Kasapis, S. (2018). Microencapsulation of fish oil with  
616 alginate: In-vitro evaluation and controlled release. *LWT - Food Science and Technology*, 90(July 2017), 310–315.  
617 <https://doi.org/10.1016/j.lwt.2017.12.045>
- 618 12. Belscak-Cvitanovic, A., Komes, D., Karlović, S., Djaković, S., Špoljarić, I., Mršić, G., & Ježek, D. (2015).  
619 Improving the controlled delivery formulations of caffeine in alginate hydrogel beads combined with pectin,  
620 carrageenan, chitosan and psyllium. *Food Chemistry*, 167, 378–386.  
621 <https://doi.org/10.1016/j.foodchem.2014.07.011>
- 622 13. Benavides, S., Cortés, P., Parada, J., & Franco, W. (2016). Development of alginate microspheres containing thyme  
623 essential oil using ionic gelation. *Food Chemistry*, 204, 77–83. <https://doi.org/10.1016/j.foodchem.2016.02.104>
- 624 14. Blanco-Cabra, N., Paetzold, B., Ferrar, T., Mazzolini, R., Torrents, E., Serrano, L., & LLuch-Senar, M. (2020).  
625 Characterization of different alginate lyases for dissolving *Pseudomonas aeruginosa* biofilms. *Scientific Reports*,  
626 10(1), 9390. <https://doi.org/10.1038/s41598-020-66293-2>
- 627 15. Blandino, A., Macías, M., & Cantero, D. (1999). Formation of calcium alginate gel capsules: Influence of sodium  
628 alginate and CaCl<sub>2</sub> concentration on gelation kinetics. *Journal of Bioscience and Bioengineering*, 88(6), 686–689.  
629 [https://doi.org/10.1016/S1389-1723\(00\)87103-0](https://doi.org/10.1016/S1389-1723(00)87103-0)
- 630 16. Bouquerand, P.-E., Dardelle, G., Erni, P., & Normand, V. (2012). An industry perspective on the advantages and  
631 disadvantages of different flavor delivery systems. In *Encapsulation Technologies and Delivery Systems for Food  
632 Ingredients and Nutraceuticals*. Elsevier Masson SAS. <https://doi.org/10.1533/9780857095909.4.453>
- 633 17. Boyd, A., & Chakrabarty, A. M. (1995). *Pseudomonas aeruginosa* biofilms: role of the alginate exopolysaccharide.  
634 *Journal of Industrial Microbiology*, 15(3), 162–168. <https://doi.org/10.1007/BF01569821>
- 635 18. Braccini, I., Grasso, R. P., & Pérez, S. (1999). Conformational and configurational features of acidic  
636 polysaccharides and their interactions with calcium ions: A molecular modeling investigation. *Carbohydrate  
637 Research*, 317(1–4), 119–130. [https://doi.org/10.1016/S0008-6215\(99\)00062-2](https://doi.org/10.1016/S0008-6215(99)00062-2)
- 638 19. Cao, L., Lu, W., Mata, A., Nishinari, K., & Fang, Y. (2020). Egg-box model-based gelation of alginate and pectin :  
639 A review. *Carbohydrate Polymers*, 242(May), 116389. <https://doi.org/10.1016/j.carbpol.2020.116389>
- 640 20. Cerciello, A., del Gaudio, P., Granata, V., Sala, M., Aquino, R. P., & Russo, P. (2017). Synergistic effect of  
641 divalent cations in improving technological properties of cross-linked alginate beads. *International Journal of  
642 Biological Macromolecules*, 101, 100–106. <https://doi.org/10.1016/j.ijbiomac.2017.03.077>
- 643 21. Chakravartula, S. S. N., Soccio, M., Lotti, N., Balestra, F., Dalla Rosa, M., & Siracusa, V. (2019). Characterization  
644 of composite edible films based on pectin/alginate/whey protein concentrate. *Materials*, 12(15), 1–19.  
645 <https://doi.org/10.3390/ma12152454>

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706
22. Chalella Mazzocato, M., Thomazini, M., & Favaro-Trindade, C. S. (2019). Improving stability of vitamin B12 (Cyanocobalamin) using microencapsulation by spray chilling technique. *Food Research International*, 126(September), 108663. <https://doi.org/10.1016/j.foodres.2019.108663>
  23. Chhatbat, M., Meena, R., Prasad, K., Siddhanta, A. K. (2009). Microwave assisted rapid method for hydrolysis of sodium alginate for M/G ratio determination. *Carbohydrate Polymers*, 76 (4), 650-656. <http://dx.doi.org/10.1016/j.carbpol.2008.11.033> .
  24. Ching, S. H., Bansal, N., & Bhandari, B. (2017). Alginate gel particles—A review of production techniques and physical properties. *Critical Reviews in Food Science and Nutrition*, 57(6), 1133–1152. <https://doi.org/10.1080/10408398.2014.965773>
  25. Chuang, J. J., Huang, Y. Y., Lo, S. H., Hsu, T. F., Huang, W. Y., Huang, S. L., & Lin, Y. S. (2017). Effects of pH on the Shape of Alginate Particles and Its Release Behavior. *International Journal of Polymer Science*, 2017. <https://doi.org/10.1155/2017/3902704>
  26. Cuadros, T. R., Skurtys, O., & Aguilera, J. M. (2012). Mechanical properties of calcium alginate fibers produced with a microfluidic device. *Carbohydrate Polymers*, 89(4), 1198–1206. <https://doi.org/10.1016/j.carbpol.2012.03.094>
  27. da Silva Fernandes, R., Tanaka, F. N., de Moura, M. R., & Aouada, F. A. (2019). Development of alginate/starch-based hydrogels crosslinked with different ions: Hydrophilic, kinetic and spectroscopic properties. *Materials Today Communications*, 21(September), 100636. <https://doi.org/10.1016/j.mtcomm.2019.100636>
  28. de Avelar, M. H. M., & Efraim, P. (2020). Alginate/pectin cold-set gelation as a potential sustainable method for jelly candy production. *Lwt*, 123(January), 109119. <https://doi.org/10.1016/j.lwt.2020.109119>
  29. de Matos, E. F., Scopel, B. S., & Dettmer, A. (2018). Citronella essential oil microencapsulation by complex coacervation with leather waste gelatin and sodium alginate. *Journal of Environmental Chemical Engineering*, 6(2), 1989–1994. <https://doi.org/10.1016/j.jece.2018.03.002>
  30. de Prisco, A., Maresca, D., Ongeng, D., & Mauriello, G. (2015). Microencapsulation by vibrating technology of the probiotic strain *Lactobacillus reuteri* DSM 17938 to enhance its survival in foods and in gastrointestinal environment. *LWT - Food Science and Technology*, 61(2), 452–462. <https://doi.org/10.1016/j.lwt.2014.12.011>
  31. Dehkordi, S. S., Alemzadeh, I., Vaziri, A. S., & Vossoughi, A. (2020). Optimization of Alginate-Whey Protein Isolate Microcapsules for Survivability and Release Behavior of Probiotic Bacteria. *Applied Biochemistry and Biotechnology*, 190(1), 182–196. <https://doi.org/10.1007/s12010-019-03071-5>
  32. Devi, N., Hazarika, D., Deka, C., & Kakati, D. K. (2012). Study of complex coacervation of gelatin a and sodium alginate for microencapsulation of olive oil. *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, 49(11), 936–945. <https://doi.org/10.1080/10601325.2012.722854>
  33. Dong, Q. Y., Chen, M. Y., Xin, Y., Qin, X. Y., Cheng, Z., Shi, L. E., & Tang, Z. X. (2013). Alginate-based and protein-based materials for probiotics encapsulation: A review. *International Journal of Food Science and Technology*, 48(7), 1339–1351. <https://doi.org/10.1111/ijfs.12078>
  34. Evageliou, V., Richardson, R. K., & Morris, E. R. (2000). Effect of pH, sugar type and thermal annealing on high-methoxy pectin gels. *Carbohydrate Polymers*, 42(3), 245–259. [https://doi.org/10.1016/S0144-8617\(99\)00191-5](https://doi.org/10.1016/S0144-8617(99)00191-5)
  35. Fang, Y., Al-Assaf, S., Phillips, G. O., & Nishinari, K. (2007). Multiple Steps and Critical Behaviors of the Binding of Calcium to Alginate. *Physical Chemistry B*, 2456–2462. <http://pubs.acs.org/doi/abs/10.1021/jp0689870%5Cnpapers2://publication/uuid/7A21EE2C-B57B-4A8F-AF4A-593AB30721B9>
  36. Fang, Z., & Bhandari, B. (2012). Spray drying, freeze drying and related processes for food ingredient and nutraceutical encapsulation. In *Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals*. Elsevier Masson SAS. <https://doi.org/10.1533/9780857095909.2.73>
  37. Feltre, G., Almeida, F. S., Sato, A. C. K., Dacanal, G. C., & Hubinger, M. D. (2020). Alginate and corn starch mixed gels: Effect of gelatinization and amylose content on the properties and in vitro digestibility. *Food Research International*, 132(February), 109069. <https://doi.org/10.1016/j.foodres.2020.109069>
  38. Feltre, G., Sartori, T., Silva, K. F. C., Dacanal, G. C., Menegalli, F. C., & Hubinger, M. D. (2020). Encapsulation of wheat germ oil in alginate-gelatinized corn starch beads: Physicochemical properties and tocopherols' stability. *Journal of Food Science*, 85(7), 2124–2133. <https://doi.org/10.1111/1750-3841.15316>
  39. Feng, L., Cao, Y., Xu, D., Wang, S., & Zhang, J. (2017). Molecular weight distribution, rheological property and structural changes of sodium alginate induced by ultrasound. *Ultrasonics Sonochemistry*, 34, 609–615. <https://doi.org/10.1016/j.ultsonch.2016.06.038>
  40. Fernandes, L., Pereira, E. L., do Céu Fidalgo, M., Gomes, A., & Ramalhosa, E. (2020). Physicochemical properties and microbial control of chestnuts (*Castanea sativa*) coated with whey protein isolate, chitosan and alginate during storage. *Scientia Horticulturae*, 263(November 2019), 109105. <https://doi.org/10.1016/j.scienta.2019.109105>
  41. Fernández Farrés, I., & Norton, I. T. (2014). Formation kinetics and rheology of alginate fluid gels produced by in-situ calcium release. *Food Hydrocolloids*, 40, 76–84. <https://doi.org/10.1016/j.foodhyd.2014.02.005>
  42. Fernández Farrés, I., Douaire, M., & Norton, I. T. (2013). Rheology and tribological properties of Ca-alginate fluid gels produced by diffusion-controlled method. *Food Hydrocolloids*, 32(1), 115–122. <https://doi.org/10.1016/j.foodhyd.2012.12.009>

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767
43. Gholamian, S., Nourani, M., & Bakhshi, N. (2021). Formation and characterization of calcium alginate hydrogel beads filled with cumin seeds essential oil. *Food Chemistry*, 338(September 2020), 128143. <https://doi.org/10.1016/j.foodchem.2020.128143>
  44. Gorji, E. G., Waheed, A., Ludwig, R., Toca-Herrera, J. L., Schleinig, G., & Gorji, S. G. (2018). Complex Coacervation of Milk Proteins with Sodium Alginate. *Journal of Agricultural and Food Chemistry*, 66(12), 3210–3220. <https://doi.org/10.1021/acs.jafc.7b03915>
  45. Gouin, S. (2004). Microencapsulation: Industrial appraisal of existing technologies and trends. *Trends in Food Science and Technology*, 15(7–8), 330–347. <https://doi.org/10.1016/j.tifs.2003.10.005>
  46. Gu, L., McClements, D. J., Li, J., Su, Y., Yang, Y., & Li, J. (2021). Formulation of alginate/carrageenan microgels to encapsulate, protect and release immunoglobulins: Egg Yolk IgY. *Food Hydrocolloids*, 112(September 2020), 106349. <https://doi.org/10.1016/j.foodhyd.2020.106349>
  47. Gu, Y., Yang, S., Zhu, G., Yuan, Y., Qu, Q., Wang, Y., & Zheng, H. (2018). The effects of cross-linking cations on the electrochemical behavior of silicon anodes with alginate binder. *Electrochimica Acta*, 269, 405–414. <https://doi.org/10.1016/j.electacta.2018.02.168>
  48. Guedes Silva, K. C., Feltre, G., Dupas Hubinger, M., & Kawazoe Sato, A. C. (2021). Protection and targeted delivery of  $\beta$ -carotene by starch-alginate-gelatin emulsion-filled hydrogels. *Journal of Food Engineering*, 290(April 2020), 110205. <https://doi.org/10.1016/j.jfoodeng.2020.110205>
  49. Günter, E. A., Popeyko, O. v., Belozarov, V. S., Martinson, E. A., & Litvinets, S. G. (2020). Physicochemical and swelling properties of composite gel microparticles based on alginate and callus cultures pectins with low and high degrees of methylesterification. *International Journal of Biological Macromolecules*, 164, 863–870. <https://doi.org/10.1016/j.ijbiomac.2020.07.189>
  50. Guo, J., Giusti, M. M., & Kaletunç, G. (2018). Encapsulation of purple corn and blueberry extracts in alginate-pectin hydrogel particles: Impact of processing and storage parameters on encapsulation efficiency. *Food Research International*, 107(February), 414–422. <https://doi.org/10.1016/j.foodres.2018.02.035>
  51. Hamed, S. F., Hashim, A. F., Abdel Hamid, H. A., Abd-Elsalam, K. A., Golonka, I., Musiał, W., & El-Sherbiny, I. M. (2020). Edible alginate/chitosan-based nanocomposite microspheres as delivery vehicles of omega-3 rich oils. *Carbohydrate Polymers*, 239(April). <https://doi.org/10.1016/j.carbpol.2020.116201>
  52. Hamedi, H., Kargozari, M., Shotorbani, P. M., Mogadam, N. B., & Fahimdanesh, M. (2017). A novel bioactive edible coating based on sodium alginate and galbanum gum incorporated with essential oil of *Ziziphora persica*: The antioxidant and antimicrobial activity, and application in food model. *Food Hydrocolloids*, 72, 35–46. <https://doi.org/10.1016/j.foodhyd.2017.05.014>
  53. Hariyadi, D. M., & Islam, N. (2020). Current Status of Alginate in Drug Delivery. *Advances in Pharmacological and Pharmaceutical Sciences*, 2020, 1–16. <https://doi.org/10.1155/2020/8886095>
  54. Harte, F., Venegas, R. (2010). A model for viscosity reduction in polysaccharides subjected to high-pressure homogenization. *Journal of Texture Studies*, 41(1), 49–61. <https://doi.org/10.1111/j.1745-4603.2009.00212.x>
  55. Hartmann, M., Dentini, M., Ingar Draget, K., & Skjåk-Bræk, G. (2006). Enzymatic modification of alginates with the manuronan C-5epimerase AlgE4 enhances their solubility at low pH. *Carbohydrate Polymers*, 63(2), 257–262. <https://doi.org/10.1016/j.carbpol.2005.08.052>
  56. Hassan, H., Gomaa, A., Subirade, M., Kheadr, E., St-Gelais, D., & Fliss, I. (2020). Novel design for alginate/resistant starch microcapsules controlling nisin release. *International Journal of Biological Macromolecules*, 153, 1186–1192. <https://doi.org/10.1016/j.ijbiomac.2019.10.248>
  57. Haug, A., Larsen, B., Samuelsson, B., Sjövall, J., & Munch-Petersen, J. (1963). The Solubility of Alginate at Low pH. In *Acta Chemica Scandinavica* (Vol. 17, pp. 1653–1662). <https://doi.org/10.3891/acta.chem.scand.17-1653>
  58. Haug, A., Larsen, B., Smidsrød, O., Munch-Petersen, J., & Munch-Petersen, J. (1963). The Degradation of Alginates at Different pH Values. *Acta Chemica Scandinavica*, 17(5), 1466–1468. <https://doi.org/10.3891/acta.chem.scand.17-1466>
  59. Hecht, H., & Srebniak, S. (2016). Structural Characterization of Sodium Alginate and Calcium Alginate. *Biomacromolecules*, 17(6), 2160–2167. <https://doi.org/10.1021/acs.biomac.6b00378>
  60. Heckert Bastos, L. P., Vicente, J., Corrêa dos Santos, C. H., Geraldo de Carvalho, M., & Garcia-Rojas, E. E. (2020). Encapsulation of black pepper (*Piper nigrum* L.) essential oil with gelatin and sodium alginate by complex coacervation. *Food Hydrocolloids*, 102(September 2019). <https://doi.org/10.1016/j.foodhyd.2019.105605>
  61. Hernández-González, A. C., Téllez-Jurado, L., & Rodríguez-Lorenzo, L. M. (2020). Alginate hydrogels for bone tissue engineering, from injectables to bioprinting: A review. *Carbohydrate Polymers*, 229(May 2019), 115514. <https://doi.org/10.1016/j.carbpol.2019.115514>
  62. Hu, M., Zheng, G., Zhao, D., & Yu, W. (2020). Characterization of the structure and diffusion behavior of calcium alginate gel beads. *Journal of Applied Polymer Science*, 137(31), 1–9. <https://doi.org/10.1002/app.48923>
  63. Jain, S., Winuprasith, T., & Suphantharika, M. (2020). Encapsulation of lycopene in emulsions and hydrogel beads using dual modified rice starch: Characterization, stability analysis and release behaviour during in-vitro digestion. *Food Hydrocolloids*, 104(January), 105730. <https://doi.org/10.1016/j.foodhyd.2020.105730>
  64. Javvaji, V., Baradwaj, A. G., Payne, G. F., Raghvan, S. R., (2011). Light-Activated Ionic Gelation of Common Biopolymers. *Langmuir* 27 (20). 12591-12596. <https://doi.org/10.1021/la201860s>



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65. Jeoh, T., Wong, D., Strobel, S., Hudnall, K., Pereira, N., Williams, K., Arbaugh, B., Cunniffe, J., & Scher, H. (2020). How alginate properties influence in situ internal gelation in Crosslinked Alginate Microcapsules (CLAMs) formed by spray-drying. *ChemRxiv*, 2. <https://doi.org/10.26434/chemrxiv.12611057>
  66. Jeong, C., Kim, S., Lee, C., Cho, S., & Kim, S. B. (2020). Changes in the physical properties of calcium alginate gel beads under a wide range of gelation temperature conditions. *Foods*, 9(2). <https://doi.org/10.3390/foods9020180>
  67. Jiao, W., Chen, W., Mei, Y., Yun, Y., Wang, B., Zhong, Q., Chen, H., & Chen, W. (2019). Effects of Molecular Weight and Gulosonic Acid/Mannuronic Acid Ratio on the Rheological Behavior and Stabilizing Property of Sodium Alginate. *Molecules* (Basel, Switzerland), 24(23), 1–12. <https://doi.org/10.3390/molecules24234374>
  68. Kabir, I. I., Sorrell, C. C., Mofarah, S. S., Yang, W., Yuen, A. C. Y., Nazir, M. T., & Yeoh, G. H. (2020). Alginate/Polymer-Based Materials for Fire Retardancy: Synthesis, Structure, Properties, and Applications. *Polymer Reviews*, 0(0), 1–58. <https://doi.org/10.1080/15583724.2020.1801726>
  69. Kelishomi, Z. H., Goliaei, B., Mahdavi, H., Nikoofar, A., Rahimi, M., Moosavi-Movahedi, A. A., Mamashli, F., & Bigdeli, B. (2016). Antioxidant activity of low molecular weight alginate produced by thermal treatment. *Food Chemistry*, 196, 897–902. <https://doi.org/10.1016/j.foodchem.2015.09.091>
  70. Kulig, D., Zimoch-Korzycka, A., Jarmoluk, A., & Marycz, K. (2016). Study on alginate-chitosan complex formed with different polymers ratio. *Polymers*, 8(5), 1–17. <https://doi.org/10.3390/polym8050167>
  71. Kurayama, F., Mohammed Bahadur, N., Furusawa, T., Sato, M., & Suzuki, N. (2020). Facile preparation of aminosilane-alginate hybrid beads for enzyme immobilization: Kinetics and equilibrium studies. *International Journal of Biological Macromolecules*, 150, 1203–1212. <https://doi.org/10.1016/j.ijbiomac.2019.10.130>
  72. Lee, B. B., Bhandari, B. R., & Howes, T. (2016). Air Extrusion System for Iontropic Alginate Microgel Particle Formation: A Review. *Chemical Engineering and Technology*, 39(12), 2355–2369. <https://doi.org/10.1002/ceat.201600088>
  73. Lee, K. Y., & Mooney, D. J. (2012). Alginate: Properties and biomedical applications. *Progress in Polymer Science* (Oxford), 37(1), 106–126. <https://doi.org/10.1016/j.progpolymsci.2011.06.003>
  74. Leirvåg, I. T. (2017). Strategies for Stabilising Calcium Alginate Gel Beads: Studies of Chitosan Oligomers, Alginate Molecular Weight and Concentration. Norwegian University of Science and Technology, May, 24.
  75. Lemos, Y. P., Mariano Marfil, P. H., & Nicoletti, V. R. (2017). Particle size characteristics of buriti oil microcapsules produced by gelatin-sodium alginate complex coacervation: Effect of stirring speed. *International Journal of Food Properties*, 20(2), 1438–1447. <https://doi.org/10.1080/10942912.2017.1349139>
  76. Leo, W. J., McLoughlin, A. J., & Malone, D. M. (1990). Effects of Sterilization Treatments on Some Properties of Alginate Solutions and Gels. *Biotechnology Progress*, 6(1), 51–53. <https://doi.org/10.1021/bp00001a008>
  77. Li, J., Wu, Y., He, J., & Huang, Y. (2016). A new insight to the effect of calcium concentration on gelation process and physical properties of alginate films. *Journal of Materials Science*, 51(12), 5791–5801. <https://doi.org/10.1007/s10853-016-9880-0>
  78. Li, L., Zhao, J., Sun, Y., Yu, F., & Ma, J. (2019). Ionically cross-linked sodium alginate/ $\kappa$ -carrageenan double-network gel beads with low-swelling, enhanced mechanical properties, and excellent adsorption performance. *Chemical Engineering Journal*, 372(February), 1091–1103. <https://doi.org/10.1016/j.cej.2019.05.007>
  79. Li, Y., Liang, M., Dou, X., Feng, C., Pang, J., Cheng, X., Liu, H., Liu, T., Wang, Y., & Chen, X. (2019). Development of alginate hydrogel/gum Arabic/gelatin based composite capsules and their application as oral delivery carriers for antioxidant. *International Journal of Biological Macromolecules*, 132, 1090–1097. <https://doi.org/10.1016/j.ijbiomac.2019.03.103>
  80. Li, Z. Q., Hou, L. da, Li, Z., Zheng, W., & Li, L. (2013). Study on shape optimization of calcium-alginate beads. *Advanced Materials Research*, 648, 125–130. <https://doi.org/10.4028/www.scientific.net/AMR.648.125>
  81. Liliana, S. C., & Vladimir, V. C. (2013). Probiotic encapsulation. *African Journal of Microbiology Research*, 7(40), 4743–4753. <https://doi.org/10.5897/ajmr2013.5718>
  82. Lim, H. P., Ooi, C. W., Tey, B. T., & Chan, E. S. (2017). Controlled delivery of oral insulin aspart using pH-responsive alginate/ $\kappa$ -carrageenan composite hydrogel beads. *Reactive and Functional Polymers*, 120(April), 20–29. <https://doi.org/10.1016/j.reactfunctpolym.2017.08.015>
  83. Liu, H., Gong, J., Chabot, D., Miller, S. S., Cui, S. W., Zhong, F., & Wang, Q. (2018). Improved survival of *Lactobacillus zeae* LB1 in a spray-dried alginate-protein matrix. *Food Hydrocolloids*, 78, 100–108. <https://doi.org/10.1016/j.foodhyd.2017.07.004>
  84. Liu, X. D., Yu, W. Y., Zhang, Y., Xue, W. M., Yu, W. T., Xiong, Y., Ma, X. J., Chen, Y., & Yuan, Q. (2002). Characterization of structure and diffusion behaviour of Ca-alginate beads prepared with external or internal calcium sources. *Journal of Microencapsulation*, 19(6), 775–782. <https://doi.org/10.1080/0265204021000022743>
  85. Lopes, M., Abraham, B., Veiga, F., Seïça, R., Cabral, L. M., Arnaud, P., Andrade, J. C., & Ribeiro, A. J. (2017). Preparation methods and applications behind alginate-based particles. *Expert Opinion on Drug Delivery*, 14(6), 769–782. <https://doi.org/10.1080/17425247.2016.1214564>
  86. Lopes, S., Bueno, L., de Aguiar Júnior, F., & Finkler, C. L. L. (2017). Preparation and characterization of alginate and gelatin microcapsules containing *Lactobacillus Rhamnosus*. *Anais Da Academia Brasileira de Ciencias*, 89(3), 1601–1613. <https://doi.org/10.1590/0001-3765201720170071>

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886  
887  
888
87. López Córdoba, A., Deladino, L., & Martino, M. (2013). Effect of starch filler on calcium-alginate hydrogels loaded with yerba mate antioxidants. *Carbohydrate Polymers*, 95(1), 315–323. <https://doi.org/10.1016/j.carbpol.2013.03.019>
  88. Lucía, C., Marcela, F., & Ainhoa, L. (2017). Encapsulation of Almond Essential Oil by Co-Extrusion/Gelling Using Chitosan as Wall Material. *Journal of Encapsulation and Adsorption Sciences*, 07(01), 67–74. <https://doi.org/10.4236/jeas.2017.71004>
  89. Mancini, F., & McHugh, T. H. (2000). Fruit-alginate interactions in novel restructured products. *Nahrung - Food*, 44(3), 152–157. [https://doi.org/10.1002/1521-3803\(20000501\)44:3<152::AID-FOOD152>3.0.CO;2-8](https://doi.org/10.1002/1521-3803(20000501)44:3<152::AID-FOOD152>3.0.CO;2-8)
  90. Martău, G. A., Mihai, M., & Vodnar, D. C. (2019). The Use of Chitosan , Alginate , and Pectin in the Biomedical and Food Sector \_ Biocompatibility, Bioadhesiveness, and Biodegradability. *Polymers*, 11, 1837. <https://doi.org/10.3390/polym11111837>.
  91. McClements, D. (2012). Requirements for food ingredient and nutraceutical delivery systems. In *Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals*. Elsevier Masson SAS. <https://doi.org/10.1533/9780857095909.1.3>
  92. McClements, David. (2017). Designing biopolymer microgels to encapsulate, protect and deliver bioactive components: Physicochemical aspects. *Advances in Colloid and Interface Science*, 240, 31–59. <https://doi.org/10.1016/j.cis.2016.12.005>
  93. Misbah, Bhatti, I. A., Zia, K. M., Bhatti, H. N., & Shahid, M. (2019). Synthesis, biological efficiency evaluation and application of sodium alginate-based polyurethane dispersions using cycloaliphatic isocyanate, as antibacterial textile coating. *Journal of Industrial Textiles*, 1528083719867445. <https://doi.org/10.1177/1528083719867445>
  94. Moe, S. T., Draget, K. I., Skjåk-Bræk, G., & Simdsrød, O. (1992). Temperature dependence of the elastic modulus of alginate gels. *Carbohydrate Polymers*, 19(4), 279–284. [https://doi.org/10.1016/0144-8617\(92\)90081-Z](https://doi.org/10.1016/0144-8617(92)90081-Z)
  95. Mohamadnia, Z., Zohuriaan-Mehr, M. J., Kabiri, K., Jamshidi, A., & Mobedi, H. (2008). Ionically cross-linked carrageenan-alginate hydrogel beads. *Journal of Biomaterials Science, Polymer Edition*, 19(1), 47–59. <https://doi.org/10.1163/156856208783227640>
  96. Mousavi, S. M. R., Rafe, A., & Yeganehzad, S. (2019). Textural, mechanical, and microstructural properties of restructured pimiento alginate-guar gels. *Journal of Texture Studies*, 50(2), 155–164. <https://doi.org/10.1111/jtxs.12385>
  97. Mousavi, S. M. R., Rafe, A., & Yeganehzad, S. (2020). Structure-rheology relationships of composite gels: Alginate and Basil seed gum/guar gum. *Carbohydrate Polymers*, 232(November 2019), 115809. <https://doi.org/10.1016/j.carbpol.2019.115809>
  98. Nayak, A. K., Das, B., & Maji, R. (2012). Calcium alginate/gum arabic beads containing glibenclamide: Development and in vitro characterization. *International Journal of Biological Macromolecules*, 51(5), 1070–1078. <https://doi.org/10.1016/j.ijbiomac.2012.08.021>
  99. Necas, J., & Bartosikova, L. (2013). Carrageenan: A review. *Veterinari Medicina*, 58(4), 187–205. <https://doi.org/10.17221/6758-VETMED>
  100. Nikoo, A. M., Kadkhodae, R., Ghorani, B., Razaq, H., & Tucker, N. (2018). Electrospray-assisted encapsulation of caffeine in alginate microhydrogels. *International Journal of Biological Macromolecules*, 116, 208–216. <https://doi.org/10.1016/j.ijbiomac.2018.04.167>
  101. Norton, I. T., Spyropoulos, F., & Cox, P. (2010). Practical Food Rheology: An Interpretive Approach. In *Practical Food Rheology: An Interpretive Approach*. <https://doi.org/10.1002/9781444391060>
  102. Oates, C. G., & Ledward, D. A. (1990). Studies on the effect of heat on alginates. *Topics in Catalysis*, 4(3), 215–220. [https://doi.org/10.1016/S0268-005X\(09\)80154-9](https://doi.org/10.1016/S0268-005X(09)80154-9)
  103. Oh, G. W., Nam, S. Y., Heo, S. J., Kang, D. H., & Jung, W. K. (2020). Characterization of ionic cross-linked composite foams with different blend ratios of alginate/pectin on the synergistic effects for wound dressing application. *International Journal of Biological Macromolecules*, 156, 1565–1573. <https://doi.org/10.1016/j.ijbiomac.2019.11.206>
  104. Okunlola, A., & Adewusi, S. A. (2019). Development of theophylline microbeads using pregelatinized breadfruit starch (*artocarpus altilis*) as a novel co-polymer for controlled release. *Advanced Pharmaceutical Bulletin*, 9(1), 93–101. <https://doi.org/10.15171/apb.2019.012>
  105. Oliveira Filho, J. G. de, Rodrigues, J. M., Valadares, A. C. F., Almeida, A. B. de, Lima, T. M. de, Takeuchi, K. P., Alves, C. C. F., Sousa, H. A. de F., Silva, E. R. da, Dyszy, F. H., & Egea, M. B. (2019). Active food packaging: Alginate films with cottonseed protein hydrolysates. *Food Hydrocolloids*, 92(January), 267–275. <https://doi.org/10.1016/j.foodhyd.2019.01.052>
  106. Oxley, J. D. (2012a). Coextrusion for food ingredients and nutraceutical encapsulation: principles and technology. In *Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals*. Elsevier Masson SAS. <https://doi.org/10.1533/9780857095909.2.131>
  107. Oxley, J. D. (2012b). Spray-cooling and spray-chilling for food ingredient and nutraceutical encapsulation. In *Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals*. Elsevier Masson SAS. <https://doi.org/10.1533/9780857095909.2.110>

- 889 108. Ozkan, G., Franco, P., de Marco, I., Xiao, J., & Capanoglu, E. (2019). A review of microencapsulation methods for  
890 food antioxidants: Principles, advantages, drawbacks and applications. *Food Chemistry*, 272(July 2018), 494–506.  
891 <https://doi.org/10.1016/j.foodchem.2018.07.205>
- 892 109. Park, D., Cheng, J., Park, J. B., Shin, S., Lee, S. H., Hong, B. H., Kim, S. H., Hyun, J., & Yang, C. (2019). PH-  
893 Triggered Silk Fibroin/Alginate Structures Fabricated in Aqueous Two-Phase System. *ACS Biomaterials Science*  
894 *and Engineering*, 5(11), 5897–5905. <https://doi.org/10.1021/acsbiomaterials.9b01216>
- 895 110. Paulo, B. B., Ramos, F. de M., & Prata, A. S. (2017). An investigation of operational parameters of jet cutting  
896 method on the size of Ca-alginate beads. *Journal of Food Process Engineering*, 40(6), 1–8.  
897 <https://doi.org/10.1111/jfpe.12591>
- 898 111. Pedrali, D., Barbarito, S., & Lavelli, V. (2020). Encapsulation of grape seed phenolics from winemaking  
899 byproducts in hydrogel microbeads – Impact of food matrix and processing on the inhibitory activity towards  $\alpha$ -  
900 glucosidase. *Lwt*, 133(June), 109952. <https://doi.org/10.1016/j.lwt.2020.109952>
- 901 112. Poncelet, D., Lencki, R., Beaulieu, C., Halle, J. P., Neufeld, R. J., & Fournier, A. (1992). Production of alginate  
902 beads by emulsification/internal gelation. I. Methodology. *Applied Microbiology and Biotechnology*, 38(1), 39–45.  
903 <https://doi.org/10.1007/BF00169416>
- 904 113. Praepanitchai, O. A., Noomhorm, A., Anal, A. K., & Potes, M. E. (2019). Survival and Behavior of Encapsulated  
905 Probiotics (*Lactobacillus plantarum*) in Calcium-Alginate-Soy Protein Isolate-Based Hydrogel Beads in Different  
906 Processing Conditions (pH and Temperature) and in Pasteurized Mango Juice. *BioMed Research International*,  
907 2019(3). <https://doi.org/10.1155/2019/9768152>
- 908 114. Puguán, J. M. C., Yu, X., & Kim, H. (2014). Characterization of structure, physico-chemical properties and  
909 diffusion behavior of Ca-Alginate gel beads prepared by different gelation methods. *Journal of Colloid and*  
910 *Interface Science*, 432, 109–116. <https://doi.org/10.1016/j.jcis.2014.06.048>
- 911 115. Qin, Y., Jiang, J., Zhao, L., Zhang, J., & Wang, F. (2018). Applications of Alginate as a Functional Food  
912 Ingredient. In *Biopolymers for Food Design*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-811449-0.00013-X>
- 913 116. Raeisi, M., Hashemi, M., Aminzare, M., Ghorbani Bidkorpeh, F., Ebrahimi, M., Jannat, B., Tepe, B., & Noori, S.  
914 M. A. (2020). Effects of Sodium Alginate and Chitosan Coating Combined with Three Different Essential Oils on  
915 Microbial and Chemical Attributes of Rainbow Trout Fillets. *Journal of Aquatic Food Product Technology*, 29(3),  
916 253–263. <https://doi.org/10.1080/10498850.2020.1722777>
- 917 117. Rahmani, V., & Sheardown, H. (2018). Protein-alginate complexes as pH/ion-sensitive carriers of proteins.  
918 *International Journal of Pharmaceutics*, 535(1–2), 452–461. <https://doi.org/10.1016/j.ijpharm.2017.11.039>
- 919 118. Rajam, R., Karthik, P., Parthasarathi, S., Joseph, G. S., & Anandharamakrishnan, C. (2012). Effect of whey protein  
920 - alginate wall systems on survival of microencapsulated *Lactobacillus plantarum* in simulated gastrointestinal  
921 conditions. *Journal of Functional Foods*, 4(4), 891–898. <https://doi.org/10.1016/j.jff.2012.06.006>
- 922 119. Rajmohan, D., & Bellmer, D. (2019). Characterization of spirulina-alginate beads formed using ionic gelation.  
923 *International Journal of Food Science*, 2019. <https://doi.org/10.1155/2019/7101279>
- 924 120. Ramos, P. E., Silva, P., Alario, M. M., Pastrana, L. M., Teixeira, J. A., Cerqueira, M. A., & Vicente, A. A. (2018).  
925 Effect of alginate molecular weight and M/G ratio in beads properties foreseeing the protection of probiotics. *Food*  
926 *Hydrocolloids*, 77, 8–16. <https://doi.org/10.1016/j.foodhyd.2017.08.031>
- 927 121. Rastegar, S., & Atrash, S. (2020). Effect of alginate coating incorporated with Spirulina, Aloe vera and guar gum  
928 on physicochemical, respiration rate and color changes of mango fruits during cold storage. *Journal of Food*  
929 *Measurement and Characterization*, 0123456789. <https://doi.org/10.1007/s11694-020-00635-6>
- 930 122. Reynolds, A. E., & Enquist, L. W. (2006). Biological interactions between herpesviruses and cyclooxygenase  
931 enzymes. *Reviews in Medical Virology*, 16(6), 393–403. <https://doi.org/10.1002/rmv.519>
- 932 123. Rezvanian, M., Ahmad, N., Mohd Amin, M. C. I., & Ng, S. F. (2017). Optimization, characterization, and in vitro  
933 assessment of alginate-pectin ionic cross-linked hydrogel film for wound dressing applications. *International*  
934 *Journal of Biological Macromolecules*, 97, 131–140. <https://doi.org/10.1016/j.ijbiomac.2016.12.079>
- 935 124. Rhein-Knudsen, N., Ale, M. T., Ajallouei, F., & Meyer, A. S. (2017). Characterization of alginates from  
936 Ghanaian brown seaweeds: *Sargassum* spp. and *Padina* spp. *Food Hydrocolloids*, 71, 236–244.  
937 <https://doi.org/10.1016/j.foodhyd.2017.05.016>
- 938 125. Rodríguez, J., Martín, M. J., Ruiz, M. A., & Clares, B. (2016). Current encapsulation strategies for bioactive oils:  
939 From alimentary to pharmaceutical perspectives. *Food Research International*, 83, 41–59.  
940 <https://doi.org/10.1016/j.foodres.2016.01.032>
- 941 126. Romo, I., Abugoch, L., & Tapia, C. (2020). Soluble complexes between chenopodins and alginate/chitosan:  
942 Intermolecular interactions and structural-physicochemical properties. *Carbohydrate Polymers*, 227(May 2019),  
943 115334. <https://doi.org/10.1016/j.carbpol.2019.115334>
- 944 127. Sabet, S., Rashidinejad, A., Melton, L. D., Zujovic, Z., Akbarinejad, A., Nieuwoudt, M., Seal, C. K., &  
945 McGillivray, D. J. (2021). The interactions between the two negatively charged polysaccharides: Gum Arabic and  
946 alginate. *Food Hydrocolloids*, 112(September 2020), 106343. <https://doi.org/10.1016/j.foodhyd.2020.106343>
- 947 128. Sagdicoglu Celep, A. G., Demirkaya, A., & Solak, E. K. (2020). Antioxidant and anticancer activities of gallic acid  
948 loaded sodium alginate microspheres on colon cancer. *Current Applied Physics*.  
949 <https://doi.org/10.1016/j.cap.2020.06.002>

- 950  
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1002  
1003  
1004  
1005  
1006  
1007  
1008  
1009
129. Salachna, P., Grzeszczuk, M., Meller, E., Soból, M. (2018). Oligo-Alginate with Low Molecular Mass Improves Growth and Physiological Activity of *Eucomis autumnalis* under Salinity Stress. *Molecules* 23 (4), 812. <https://doi.org/10.3390/molecules23040812>.
  130. Sanchez-Ballester, N. M., Soulaïrol, I., Bataille, B., & Sharkawi, T. (2019). Flexible heteroionic calcium-magnesium alginate beads for controlled drug release. *Carbohydrate Polymers*, 207(November 2018), 224–229. <https://doi.org/10.1016/j.carbpol.2018.11.096>
  131. Saravanan, M., & Rao, K. P. (2010). Pectin-gelatin and alginate-gelatin complex coacervation for controlled drug delivery: Influence of anionic polysaccharides and drugs being encapsulated on physicochemical properties of microcapsules. *Carbohydrate Polymers*, 80(3), 808–816. <https://doi.org/10.1016/j.carbpol.2009.12.036>
  132. Senturk Parreidt, T., Müller, K., Schmid, M. (2018). Alginate-Based Edible Films and Coatings for Food Packaging Applications. *Foods*, 7(10):170. <https://doi.org/10.3390/foods7100170>
  133. Shi, S. S., Lei, S. J., & Fu, C. xia. (2020). Studies of the properties of CHG-Loaded alginate fibers for medical application. *Polymer Testing*, 83(September 2019), 106141. <https://doi.org/10.1016/j.polymertesting.2019.106141>
  134. Silva, M. P., Tolini, F. L., Martins, E., Penning, M., Fávares-Trindade, C. S., & Poncelet, D. (2018). Comparison of extrusion and co-extrusion encapsulation techniques to protect *Lactobacillus acidophilus* LA3 in simulated gastrointestinal fluids. *LWT - Food Science and Technology*, 89(June 2017), 392–399. <https://doi.org/10.1016/j.lwt.2017.11.008>
  135. Sonogo, J. M., Santagapita, P. R., Perullini, M., & Jobbágy, M. (2016). Ca(II) and Ce(III) homogeneous alginate hydrogels from the parent alginic acid precursor: A structural study. *Dalton Transactions*, 45(24), 10050–10057. <https://doi.org/10.1039/c6dt00321d>
  136. Spadari, C. de C., de Bastiani, F. W. M. da S., Lopes, L. B., & Ishida, K. (2019). Alginate nanoparticles as non-toxic delivery system for miltefosine in the treatment of candidiasis and cryptococcosis. *International Journal of Nanomedicine*, 14, 5187–5199. <https://doi.org/10.2147/IJN.S205350>
  137. Stokke, B. T., Draget, K. I., Smidsrød, O., Yuguchi, Y., Urakawa, H., & Kajiwar, K. (2000). Small-angle X-ray scattering and rheological characterization of alginate gels. 1. Ca-alginate gels. *Macromolecules*, 33(5), 1853–1863. <https://doi.org/10.1021/ma991559q>
  138. Strasdat, B., & Bunjes, H. (2013). Incorporation of lipid nanoparticles into calcium alginate beads and characterization of the encapsulated particles by differential scanning calorimetry. *Food Hydrocolloids*, 30(2), 567–575. <https://doi.org/10.1016/j.foodhyd.2012.07.013>
  139. Strobel, S. A., Allen, K., Roberts, C., Jimenez, D., Scher, H. B., & Jeoh, T. (2018). Industrially-Scalable Microencapsulation of Plant Beneficial Bacteria in Dry Cross-Linked Alginate Matrix. *Industrial Biotechnology*, 14(3), 138–147. <https://doi.org/10.1089/ind.2017.0032>
  140. Strobel, S. A., Hudnall, K., Arbaugh, B., Cunniffe, J. C., Scher, H. B., & Jeoh, T. (2020). Stability of Fish Oil in Calcium Alginate Microcapsules Cross-Linked by In Situ Internal Gelation During Spray Drying. *Food and Bioprocess Technology*, 13(2), 275–287. <https://doi.org/10.1007/s11947-019-02391-y>
  141. Su, C., Feng, Y., Ye, J., Zhang, Y., Gao, Z., Zhao, M., Yang, N., Nishinari, K., & Fang, Y. (2018). Effect of sodium alginate on the stability of natural soybean oil body emulsions. *RSC Advances*, 8(9), 4731–4741. <https://doi.org/10.1039/c7ra09375f>
  142. Su, Y., Gu, L., Zhang, Z., Chang, C., Li, J., McClements, D. J., & Yang, Y. (2019). Encapsulation and release of egg white protein in alginate microgels: Impact of pH and thermal treatment. *Food Research International*, 120(February), 305–311. <https://doi.org/10.1016/j.foodres.2019.02.048>
  143. Sun, Q., Zhang, Z., Zhang, R., Gao, R., & McClements, D. J. (2018). Development of Functional or Medical Foods for Oral Administration of Insulin for Diabetes Treatment: Gastroprotective Edible Microgels. *Journal of Agricultural and Food Chemistry*, 66(19), 4820–4826. <https://doi.org/10.1021/acs.jafc.8b00233>
  144. Sun, X., Cameron, R. G., & Bai, J. (2020). Effect of spray-drying temperature on physicochemical, antioxidant and antimicrobial properties of pectin/sodium alginate microencapsulated carvacrol. *Food Hydrocolloids*, 100(October 2019), 105420. <https://doi.org/10.1016/j.foodhyd.2019.105420>
  145. Taha, A., Ahmed, E., Ismaeil, A., Ashkkumar, M., Xu, X., Pan, S., Hu, H. (2020). Ultrasonic emulsification: An overview on the preparation of different emulsifiers-stabilized emulsions. *Trends in Food Science & Technology*, 105 (September), 363–377. Elsevier Inc. <https://doi.org/10.1016/j.tifs.2020.09.024>.
  146. Taqieddin, E., & Amiji, M. (2004). Enzyme immobilization in novel alginate-chitosan core-shell microcapsules. *Biomaterials*, 25(10), 1937–1945. <https://doi.org/10.1016/j.biomaterials.2003.08.034>
  147. Tavassoli-Kafrani, E., Shekarchizadeh, H., & Masoudpour-Behabadi, M. (2016). Development of edible films and coatings from alginates and carrageenans. *Carbohydrate Polymers*, 137, 360–374. <https://doi.org/10.1016/j.carbpol.2015.10.074>
  148. Thies, C. (2012). Microencapsulation methods based on biopolymer phase separation and gelation phenomena in aqueous media. In *Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals*. Elsevier Masson SAS. <https://doi.org/10.1533/9780857095909.2.177>
  149. Topuz, F., Henke, A., Richter, W., & Groll, J. (2012). Magnesium ions and alginate do form hydrogels: A rheological study. *Soft Matter*, 8(18), 4877–4881. <https://doi.org/10.1039/c2sm07465f>

- 1010 150. Tsai, F. H., Kitamura, Y., & Kokawa, M. (2017). Effect of gum arabic-modified alginate on physicochemical  
 1011 properties, release kinetics, and storage stability of liquid-core hydrogel beads. *Carbohydrate Polymers*, 174, 1069–  
 1012 1077. <https://doi.org/10.1016/j.carbpol.2017.07.031>
- 1013 151. van Leusden, P., den Hartog, G. J. M., Bast, A., Postema, M., van der Linden, E., & Sagis, L. M. C. (2017).  
 1014 Permeation of probe molecules into alginate microbeads: Effect of salt and processing. *Food Hydrocolloids*, 73,  
 1015 255–261. <https://doi.org/10.1016/j.foodhyd.2017.06.028>
- 1016 152. Vaziri, A. S., Alemzadeh, I., Vossoughi, M., & Khorasani, A. C. (2018). Co-microencapsulation of *Lactobacillus*  
 1017 *plantarum* and DHA fatty acid in alginate-pectin-gelatin biocomposites. *Carbohydrate Polymers*, 199(July), 266–  
 1018 275. <https://doi.org/10.1016/j.carbpol.2018.07.002>
- 1019 153. Vinet, L., & Zhedanov, A. (2011). A “missing” family of classical orthogonal polynomials. In B. H. A. Rehm  
 1020 (Ed.), *Journal of Physics A: Mathematical and Theoretical* (Vol. 44, Issue 8, pp. 7250–7257).  
 1021 <https://doi.org/10.1088/1751-8113/44/8/085201>
- 1022 154. Vold, I. M. N., Kristiansen, K. A., & Christensen, B. E. (2006). A study of the chain stiffness and extension of  
 1023 alginates, in vitro epimerized alginates, and periodate-oxidized alginates using size-exclusion chromatography  
 1024 combined with light scattering and viscosity detectors. *Biomacromolecules*, 7(7), 2136–2146.  
 1025 <https://doi.org/10.1021/bm060099n>
- 1026 155. Vu, H. C., Dwivedi, A. D., Le, T. T., Seo, S. H., Kim, E. J., & Chang, Y. S. (2017). Magnetite graphene oxide  
 1027 encapsulated in alginate beads for enhanced adsorption of Cr(VI) and As(V) from aqueous solutions: Role of  
 1028 crosslinking metal cations in pH control. *Chemical Engineering Journal*, 307, 220–229.  
 1029 <https://doi.org/10.1016/j.cej.2016.08.058>
- 1030 156. Wang, W., Waterhouse, G. I. N., & Sun-Waterhouse, D. (2013). Co-extrusion encapsulation of canola oil with  
 1031 alginate: Effect of quercetin addition to oil core and pectin addition to alginate shell on oil stability. *Food Research*  
 1032 *International*, 54(1), 837–851. <https://doi.org/10.1016/j.foodres.2013.08.038>
- 1033 157. Warsinger, D. M., Gonzalez, J. v., Belleghem, S. M. van, Servi, A., Swaminathan, J., & Lienhard, J. H. (2015). The  
 1034 Combined Effect of Air Layers and Membrane Superhydrophobicity on Biofouling in Membrane Distillation. The  
 1035 American Water Works Association Annual Conference and Exposition, June, 0–14.  
 1036 <http://hdl.handle.net/1721.1/112263>.
- 1037 158. Wong, T., Chan, L., Kho, S., Sia Heng, O. (2002). Design of controlled-release solid dosage forms of alginate and  
 1038 chitosan using microwave. *Journal of controlled release*, 84 (3), 99-114. [https://doi-org.bases-doc.univ-](https://doi-org.bases-doc.univ-lorraine.fr/10.1016/S0168-3659(02)00237-7)  
 1039 [lorraine.fr/10.1016/S0168-3659\(02\)00237-7](https://doi-org.bases-doc.univ-lorraine.fr/10.1016/S0168-3659(02)00237-7)
- 1040 159. Wu, Y., Weller, C. L., Hamouz, F., Cuppett, S., & Schnepf, M. (2001). Moisture loss and lipid oxidation for  
 1041 precooked ground-beef patties packaged in edible starch-alginate-based composite films. *Journal of Food Science*,  
 1042 66(3), 486–493. <https://doi.org/10.1111/j.1365-2621.2001.tb16137.x>
- 1043 160. Yang, J. S., Xie, Y. J., & He, W. (2011). Research progress on chemical modification of alginate: A review.  
 1044 *Carbohydrate Polymers*, 84(1), 33–39. <https://doi.org/10.1016/j.carbpol.2010.11.048>
- 1045 161. Yang, J., Steck, J., & Suo, Z. (2020). Gelation kinetics of alginate chains through covalent bonds. *Extreme*  
 1046 *Mechanics Letters*, 40, 100898. <https://doi.org/10.1016/j.eml.2020.100898>
- 1047 162. Yin, Y., & Cadwallader, K. R. (2018). Spray-chilling encapsulation of 2-acetyl-1-pyrroline zinc chloride complex  
 1048 using hydrophobic materials: Feasibility and characterization of microcapsules. *Food Chemistry*, 265(February),  
 1049 173–181. <https://doi.org/10.1016/j.foodchem.2018.05.079>
- 1050 163. Zazzali, I., Aguirre Calvo, T. R., Pizones Ruíz-Henestrosa, V. M., Santagapita, P. R., & Perullini, M. (2019).  
 1051 Effects of pH, extrusion tip size and storage protocol on the structural properties of Ca(II)-alginate beads.  
 1052 *Carbohydrate Polymers*, 206(November 2018), 749–756. <https://doi.org/10.1016/j.carbpol.2018.11.051>
- 1053 164. Zhang, B., Hu, B., Nakauma, M., Funami, T., Nishinari, K., Draget, K. I., Phillips, G. O., & Fang, Y. (2019).  
 1054 Modulation of calcium-induced gelation of pectin by oligogulonate as compared to alginate. *Food Research*  
 1055 *International*, 116(August 2018), 232–240. <https://doi.org/10.1016/j.foodres.2018.08.020>
- 1056 165. Zhang, H., Zhang, F., & Yuan, R. (2019). Applications of natural polymer-based hydrogels in the food industry. In  
 1057 *Hydrogels Based on Natural Polymers* (pp. 357–410). Elsevier Inc. [https://doi.org/10.1016/B978-0-12-816421-](https://doi.org/10.1016/B978-0-12-816421-1.00015-X)  
 1058 [1.00015-X](https://doi.org/10.1016/B978-0-12-816421-1.00015-X)