

# *Encapsulation of lactobacillus casei into calcium pectinate-chitosan beads for enteric delivery*

Article

Accepted Version

Bepeyeva, A., de Barros, J. M. S., Albadran, H., Kakimov, A. K., Kakimova, Z. K., Charalampopoulos, D. ORCID: <https://orcid.org/0000-0003-1269-8402> and Khutoryanskiy, V. ORCID: <https://orcid.org/0000-0002-7221-2630> (2017) Encapsulation of lactobacillus casei into calcium pectinate-chitosan beads for enteric delivery. *Journal of Food Science*, 82 (12). pp. 2954-2959. ISSN 0022-1147 doi: <https://doi.org/10.1111/1750-3841.13974> Available at <https://centaur.reading.ac.uk/73925/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

Published version at: <http://onlinelibrary.wiley.com/doi/10.1111/1750-3841.13974/full>

To link to this article DOI: <http://dx.doi.org/10.1111/1750-3841.13974>

Publisher: Wiley

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

[www.reading.ac.uk/centaur](http://www.reading.ac.uk/centaur)

**CentAUR**

Central Archive at the University of Reading

Reading's research outputs online

1                   **Encapsulation of *Lactobacillus casei* into calcium pectinate-chitosan**  
2                   **beads for enteric delivery**

3  
4                   Aigerim Bepeyeva<sup>a</sup>, Joao M.S. de Barros<sup>b</sup>, Hanady Albadran<sup>c</sup>, Aitbek K. Kakimov<sup>a</sup>,  
5                   Zhaynagul Kh. Kakimova<sup>a</sup>, Dimitris Charalampopoulos<sup>c</sup>, Vitaliy V. Khutoryanskiy<sup>b\*</sup>  
6

7                   <sup>a</sup> Department of Standardization and Biotechnology, Shakarim State University of Semey, Semey,  
8                   VKO, Kazakhstan

9                   <sup>b</sup> Reading School of Pharmacy, University of Reading, Whiteknights, Reading, Berkshire, PO Box  
10                  224, RG6 6AD, United Kingdom

11                  <sup>c</sup> Department of Food and Nutritional Sciences, University of Reading, Whiteknights, Reading,  
12                  Berkshire, RG6 6AD United Kingdom

13  
14                  \*Corresponding author at: Reading School of Pharmacy, University of Reading, Whiteknights,  
15                  Reading, Berkshire, PO Box 224, RG6 6AD, United Kingdom. Tel.: + +44 (0) 118 378 6119; fax:  
16                  +44 (0) 118 378 4703.

17                  E-mail address: v.khutoryanskiy@reading.ac.uk (Prof Vitaliy V. Khutoryanskiy).  
18  
19

20                  **ABSTRACT**

21                  Gel beads were prepared by extrusion of various types of pectin into 0.15 M calcium chloride. Size,  
22                  morphology, and textural properties of three types of beads were evaluated and it was established that  
23                  the use of 3 w/v % amidated pectin provides the optimal characteristics suitable for encapsulation of  
24                  live bacteria. *Lactobacillus casei* NCIMB 30185 (PXN37) was encapsulated into calcium pectinate gel  
25                  through the extrusion of a live bacteria dispersion in 3 w/v % pectin into a solution of calcium  
26                  chloride. The capsules were then additionally coated with chitosan. The viability of bacteria within  
27                  these capsules was studied under model gastrointestinal conditions in vitro (simulated gastric and  
28                  intestinal juices). It was established that pectin–chitosan capsules can provide protection to  
29                  *Lactobacillus casei* from the gastric acid and result in high levels of viable bacteria released in the  
30                  intestine.

31  
32                  **Practical Application:**

33                  Encapsulation of *Lactobacillus casei* into calcium pectinate beads coated with chitosan provided  
34                  capsules capable of delivery live probiotic bacteria into the intestine.  
35  
36

37  
38  
39 *Key words:*  
40 Encapsulation  
41 *Lactobacillus casei*  
42 Pectin  
43 Chitosan  
44 Probiotic  
45 Live bacteria  
46

## 47 **1. Introduction**

48

49 Gastrointestinal delivery of live probiotic bacteria is considered as a promising approach to  
50 improve the gut health. Probiotics provide some health benefits due to their ability to facilitate  
51 digestion, produce vitamins, bust the immune system and prevent the growth of pathogenic bacteria  
52 (Derrien and others 2015; Reid and others 2016).

53 Probiotics are typically found in some dairy products; however, their successful delivery to the gut  
54 is often compromised because of the sensitivity of these bacteria to the acidic environment in the  
55 stomach (Burgain and others 2011; Cook and others 2012). Encapsulation of live probiotics into  
56 materials resistant to gastric acid is a viable strategy for their successful oral delivery. Previously, we  
57 have reported the use of calcium alginate beads coated with chitosan for encapsulation and oral  
58 delivery of *Bifidobacterium* species (Cook and others 2011; Cook and others 2013; Cook and others  
59 2013; Nualkaekul and others 2013). It was established that the live bacteria encapsulated within  
60 calcium alginate beads coated with chitosan may potentially survive the transit through the harsh  
61 environment of the stomach and release high levels of live probiotic in the intestine. The protective  
62 effect of chitosan coating was found to be due to its ability to delay acid diffusion into the capsules  
63 (Cook and others 2013). It was also demonstrated that live probiotic bacteria encapsulated within  
64 calcium alginate-chitosan show excellent survival in acidic juices (Nualkaekul and others 2013) and  
65 during long storage in dry state (Albadran and others 2015).

66 Pectins is a group of anionic polysaccharides present in many fruits and vegetables and have a  
67 wide range of food and pharmaceutical applications as gelling, thickening, and stabilizing agents  
68 (Thakur, Singh, Handa & Rao, 1997). They consist of several types of carbohydrate repeating units,  
69 including homogalacturonan (HG) and type I rhamnogalacturonan (RG-I). HG regions in pectins  
70 consist of poly-galacturonic acid residues; some of these are partially methyl esterified. Pectins with a  
71 high degree of methyl substitution (> 50%) are classified as high methyl ester pectins and those with <  
72 50 % esterification are called low methyl ester pectins. Similarly to alginates, pectins are capable of  
73 forming gels upon extrusion of their aqueous solutions into the media containing soluble calcium salts

74 (Morris et al, 2010). Pectin properties can also be modified by their reaction with ammonia under  
75 alkaline conditions resulting in amidated pectins. Low methyl ester amidated pectins typically require  
76 lower  $\text{Ca}^{2+}$  concentration to form gels and are more tolerant than low methyl ester pectin with respect  
77 to excess calcium concentrations (Belitz and others 2004).

78 Previously, pectins were reported as materials for encapsulation of *Lactobacillus rhamnosus*  
79 (Gerez and others 2012) and *Lactobacillus acidophilus* (Gebara and others 2013) with the subsequent  
80 coating of these capsules with whey proteins in both cases. This coating provided a protective function  
81 that ensured the effective transit of live bacteria through the gastrointestinal tract. Pectins were also  
82 used for encapsulation of probiotics in combination with other materials such as alginates (Sandoval-  
83 Castilla and others 2010), hyaluronic acid (Pliszcak and others 2011) and milk (Shi and others 2013).  
84 Typically the use of alginates and pectins for encapsulation of probiotics give excellent encapsulation  
85 efficiencies of 90-100 % (Shori, 2017).

86 In this work the ability of different pectins to form calcium gel capsules was evaluated and the  
87 most promising materials were used for encapsulation of *Lactobacillus casei*. The gel capsules with  
88 live probiotic were then coated with chitosan. In vitro experiments were also performed with the  
89 encapsulated probiotic bacteria to establish the protective role of chitosan coating for the successful  
90 transit of live bacteria through the gastrointestinal tract.

91

## 92 **2. Materials and methods**

93

### 94 2.1 Materials

95

96 Amidated pectin CU 020 (63 kDa, degrees of esterification and amidation are 28 % and 20 %,   
97 respectively), low methyl ester pectin Classic CU 701 (54 kDa and degree of esterification 36 %), high   
98 methyl pectin Classic CU-L 004/14 (67 kDa and degree of esterification 59 %) were obtained from   
99 HERBSTREITH & Fox KG (Germany). Chitosan (103 kDa and degree of deacetylation 85.6%), and   
100 fluorescein isothiocyanate (FITC) (isomer I) were purchased from Sigma-Aldrich (Gillingham, U.K.).   
101 Fluorescently-labelled chitosan was synthesized according to the methodology described by our   
102 research group previously (Cook, 2011).  $\text{CaCl}_2$  was purchased from Fisher Scientific UK Limited. The   
103 microbiology media Man, Rogosa and Sharpe (MRS) broth, agar, and phosphate-buffered saline (PBS)   
104 were purchased from Oxoid (Basingstoke, UK). Pectin solutions and chitosan solution were purified   
105 by microfiltration (0.45  $\mu\text{m}$ , Minisart filter, Sartorius Stedim, Biotech). All other reagents and   
106 materials were sterilized in an autoclave (121°C, 15 min). *Lactobacillus casei* NCIMB 30185 (PXN   
107 37) strain was received from Probiotics International Ltd (Protexin) (Somerset, UK).

108  
109  
110  
111  
112  
113  
114  
115  
116  
  
117  
118  
119  
120  
121  
122  
123  
124  
125  
126  
127  
128  
129  
130  
131  
132  
133  
134  
135  
136  
137  
138  
139  
140  
141

## 2.2 Culture preparation

*Lactobacillus casei* NCIMB 30185 (1XN37) was spread onto MRS agar from a cell bank and was incubated at 37°C for 48 h. A colony was picked and inoculated into MRS broth (10 mL) and incubated at 37 °C for 24 h to get a bacterial suspension with an OD<sub>600</sub> of approximately 2.0. After growth the cells were separated by centrifugation for 10 min (3200 rpm, at 4°C), the supernatant was collected, the cell pellet was rinsed with 1 mL PBS and redispersed in 10 mL pectin solution to give approximately  $6 \times 10^9$  CFU/mL.

## 2.3 Preparation of unloaded pectin capsules

Unloaded (without *L. casei*) pectin capsules were prepared using an extrusion method. 1, 2, and 3 w/v % pectin aqueous solutions (1 mL) were extruded with a syringe (0.8 mm diameter needle) and a pump (flow rate set at 2 mL min<sup>-1</sup>) into 50 mL 0.15 M CaCl<sub>2</sub>. Gel beads were formed upon contact with CaCl<sub>2</sub>, and were left to harden for 30 min. The distance from the surface of the solution and the needle was 10 cm. After hardening, the pectin gel beads were removed from the solution *via* filtration. Chitosan coated capsules were prepared by dispersing pectin gel beads in 10 mL chitosan solution (0.4% w/v in 0.1 M acetic acid; pH set to 6 using 1M microfiltered NaOH,) under gentle stirring for 10 minutes and then subsequently were removed by filtration.

## 2.4 Production of probiotic containing pectin capsules

In order to produce probiotic containing capsules, the *L. casei* suspension was mixed with 3% amidated pectin solution (1:9 volume ratio) and the capsules were generated by extrusion and coated as described above. The viability and bacteria release studies are described in terms of quantity of extruded pectin (each batch of capsules corresponds to 1 mL of extruded pectin solution).

## 2.5 Morphology of the capsules prepared from grades of pectin

The morphology of the gel beads prepared using various types and different concentrations of pectin (1, 2, and 3 w/v % of amidated, low methyl ester and high methyl ester pectins) was evaluated with a light microscope (Leica DM2500). Gel beads were examined under  $\times 1.6$ -2.5 magnification.

## 2.6 Mechanical properties and dimensions of gel beads

142

143 The diameters of uncoated beads were determined with a light microscope (Leica DM2500) and  
144 using an image analysis software (ImageJ). The compressive strength was determined using Texture  
145 Analyser (Stable Microsystems, UK), with a P\1K steel probe at 1 mm s<sup>-1</sup> rate, with 0.01 N trigger  
146 force.

147

148

149

150

## 151 2.7 Viability of *Lactobacillus casei* in simulated gastric juice (SGJ)

152

153 For non-encapsulated bacteria, *L. casei* was inoculated in MRS broth (10 mL) and the culture  
154 incubated at 37°C for 24 hours. It was then separated by centrifugation for 10 min at 4 °C (3200 rpm);  
155 the bacteria were collected and rinsed with PBS (1 mL). Following the incubation, a 1 mL aliquot was  
156 taken to evaluate the initial cell concentration using the spread plating method. Cells were diluted with  
157 PBS, and 0.1 mL of the appropriate dilution was spread onto MRS agar. Plates were incubated at 37  
158 °C for 48 h and the colony forming units (CFU) were determined. To evaluate cell survival in gastric  
159 conditions, SGJ (10 mL, pH 2.0) was added to cells and incubated at 37°C with shaking at 100 rpm.  
160 After 15 min, 30 min, 60 min and 120 min intervals an aliquot (1 mL) of the SGJ was diluted in 9 mL  
161 PBS and enumerated using spread plating method described above.

162 For encapsulated bacteria, 3 batches with *L. casei* were prepared from the same broth of cells.  
163 Two of these batches were transferred into SGJ and incubated at 37 °C with shaking at 100 rpm. The  
164 third batch was transferred into 100 mL of PBS and incubated for 1 h at 37 °C with shaking at 100  
165 rpm. These batches were then homogenized in Seward stomach 400 circulator for 20 min at 230 rpm)  
166 and enumerated to give the initial bacteria concentration. The two batches of beads in the SGJ were  
167 removed after 1 and 2 h of incubation. These beads were placed into 100 mL of PBS and incubated for  
168 1 h at 37 °C with shaking at 100 rpm. Then the beads were dissolved and enumerated using the  
169 methods described above.

170

## 171 2.8 Release of bacteria from capsules

172

173 The release of bacteria from the capsules was studied during 120 min of incubation the  
174 capsules in SGJ for 2 h (pH 2.0, 10 mL at 37°C with shaking at 100 rpm); then these were removed *via*  
175 filtration and placed into simulated intestinal juice for 3 h (pH 7.2, 100 mL at 37°C with shaking at

176 100 rpm). Then this mixture was transferred into Seward stomach 400 circulator for 20 min (230 rpm)  
177 and the bacteria counted by taking 1 mL aliquots. 1mL aliquots were also taken after 1 and 2 hours in  
178 SGJ and after 1 and 2 hours in SIJ in order plot the bacteria release profiles. The enumeration of viable  
179 bacteria was performed the spread plating method.

180

## 181 2.9 Determination of coat thickness using fluorescent microscopy

182

183 The thickness of the chitosan coat was determined by fluorescent microscopy using a Leica  
184 DM2500 microscope. The lyophilized FITC-chitosan was dissolved in 0.1 M acetic acid to form 0.4%  
185 (w/v) solution, with pH adjusted to 6.0 using 1 M NaOH. This was then used to coat pectin beads as  
186 described in section 2.3. The bead images were taken using a Leica DM2500 fluorescent stereo-  
187 microscope. The thickness of coating layer was determined using ImageJ.

188

## 189 2.10 Statistical analysis

190

191 Statistical analysis was conducted with a two-tailed unpaired Student's t-test and  $p < 0.05$  was  
192 considered as significant.

193

# 194 3. Results and discussion

195

## 196 3.1 Morphology, diameter and texture properties of beads

197

198 The influence of pectin concentration on the formation of gel beads was initially investigated.  
199 Three different types of pectin were studied as potential materials to form calcium pectinate gel  
200 capsules (amidated pectin, low methyl pectin and high methyl pectin). It was observed that the  
201 extrusion of these solutions into 0.15 M  $\text{CaCl}_2$  results in the formation of gel beads of different shape  
202 and it depends on the type of pectin used as well as the concentration of its solution (Fig. 1). More  
203 spherical beads are formed upon increasing the polymer concentration, which is possibly related to an  
204 increase in solution viscosity.

205

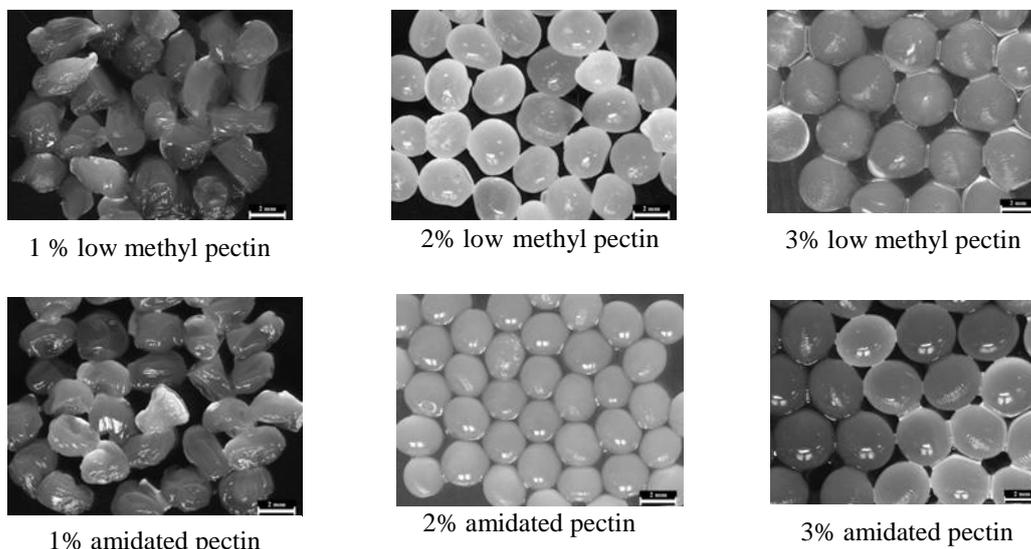
206

207

208

209

210  
211  
212  
213  
214  
215  
216  
217



218

219 Fig.1. Morphology of capsules prepared with different types and concentration of pectin. A 1.6-  
220 2.5 $\times$ magnification was used. The scale bar represents 2 mm.

221

222 It was noted that encapsulation with 1% (w/v) pectin does not give a spherical shape to the  
223 capsules. Furthermore, when 1% (w/v) high methyl pectin was used it was observed that capsule  
224 formation did not occur. This can be due to the fact that the gelling properties of pectin depend on the  
225 degree of esterification (Soumya & Suvendu, 2012). It was previously reported that high methyl  
226 pectins will form gel in the presence of sugars or other small molecules (e.g. polyols or monohydric  
227 alcohols) (Oakenfull & Scott, 1984; Thakur, Singh & Handa, 1997), and at low pH (3.0–4.5).  
228 Additionally, gelation of low methyl pectins takes place solely in the presence of divalent cations such  
229 as calcium (Soumya & Suvendu, 2012).

230 Table 1 presents a summary of the key physicochemical characteristics (diameter and  
231 compressive strength) of the gel beads from the different pectins used. Note that size and mechanical  
232 strength were characterised only for spherical beads.

233

234

235

236 **Table 1.** Physical characteristics of pectin beads

Polymer	Concentration, % (w/v)	Compressive strength, N	Diameter of beads, mm	Comments
Low methyl pectin	1	-	-	Capsules are not spherical
High methyl pectin	1	-	-	Do not form capsules
Amidated pectin	1	-	-	Capsules are not spherical
Low methyl pectin	2	2.12 $\pm$ 0.34	2.57 $\pm$ 0.02	Spherical capsules

High methyl pectin	2	-	-	Do not form capsules
Amidated pectin	2	8.88±0.57	2.76±0.01	Spherical capsules
Low methyl pectin	3	3.32±0.29	3.47±0.03	Spherical capsules
Amidated pectin	3	9.37±0.56	3.50±0.05	Spherical capsules
High methyl pectin	3	-	-	Do not form capsules

237

238

239 As expected, it was found that with increasing the concentration of pectin, the size and  
 240 mechanical strength of the capsules also increased. Amidated pectin also formed mechanically  
 241 stronger capsules compared to low methyl ester pectin at the same polymer concentration. According  
 242 to the literature (Chandramouli and others 2004; Sabikhi and others 2010; Hansen and others 2002)  
 243 larger capsules typically provide better protection to cells towards adverse environmental conditions.  
 244 Spheres with size ranges between 1 and 3 mm are preferably used in immobilised cell technology  
 245 applications (Heidebach and others 2012). Anal and Singh (2007) also stated that the large size of  
 246 bacteria (typically 1-4  $\mu\text{m}$ ) is a serious challenge for cell encapsulation. These dimensional  
 247 characteristics limit cell loading in small capsules, and in the case that larger capsules they will have  
 248 suboptimal textural and sensorial properties in food products. Muthukumarasamy et al. (2006)  
 249 established that larger capsules (2-4 mm) improved the viability of *Lactobacillus reuteri* compared to  
 250 smaller capsules (20-1000  $\mu\text{m}$ ). Chandramouli et al. (2004) also demonstrated that the viability of  
 251 lactobacilli in simulated gastric conditions was greater for alginate capsules of larger size (200-1000  
 252  $\mu\text{m}$ ). Due to their larger size and greater mechanical strength, amidated pectin (3% (w/v)) was selected  
 253 for further work involving the encapsulation of *Lactobacillus casei*.

254

255

256

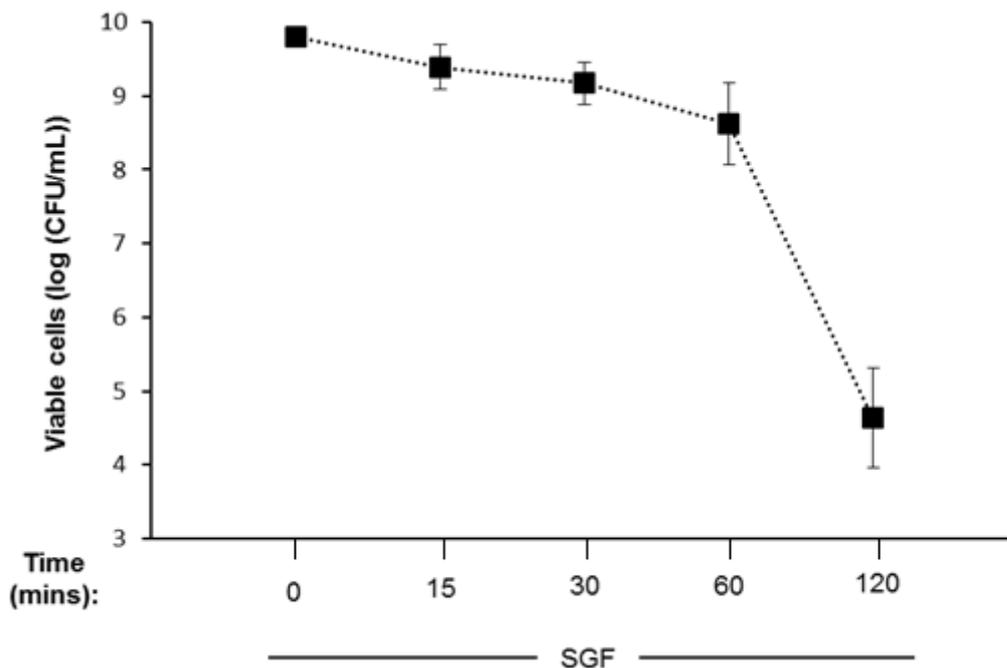
### 257 3.2 Viability of non-encapsulated *Lactobacillus casei* in SGJ

258

259 The viability of free *L. casei* in SGJ (pH 2.0) was evaluated to establish if these cells were acid  
 260 resistant. After 60 minutes of SGJ exposure a reduction in cell viability of 1 log was observed (Fig. 2)  
 261 and after 120 min even greater decrease in cell numbers of approximately 5 logs was seen, giving a  
 262 final cell recovery of  $10^5$  CFU/mL. This indicates that *L. casei* is sensitive to the acidic environment in  
 263 the stomach and there is a need for an encapsulation system to protect *L. casei* cells during its  
 264 gastrointestinal delivery.

265

266



267

268

269 Fig.2. Viability of free *Lactobacillus casei* in SGJ (pH 2.0), n=3.

270

271

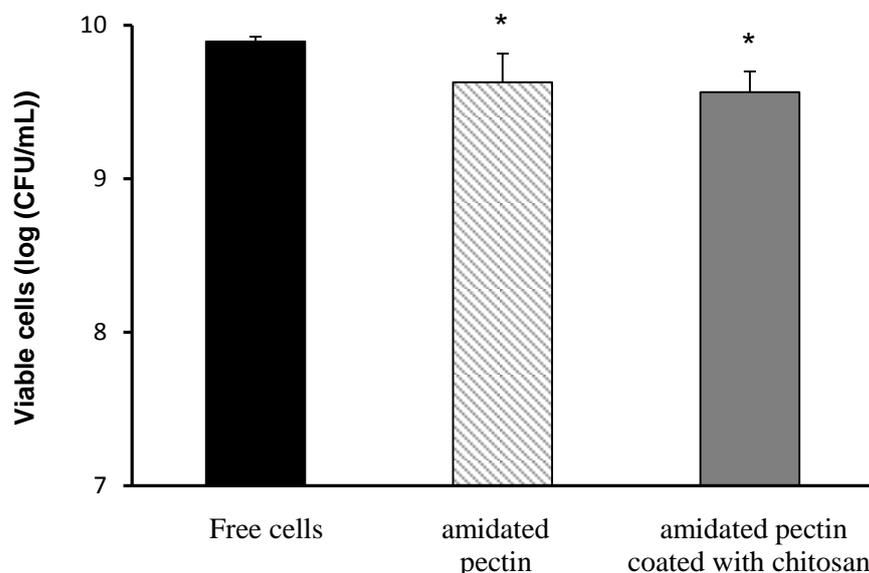
### 272 3.3 Viability of *Lactobacillus casei* during its encapsulation into calcium pectinate beads 273 and subsequent coating with chitosan

274

275 The effect of encapsulation within calcium pectinate gel and the effect of coating with chitosan  
276 on the viability of *L. casei* were investigated. It was established that the encapsulation process resulted  
277 in approximately 0.3 log CFU/mL reduction in cell viability (Fig.3). However, there was no loss in  
278 viability associated with the coating process. A similar small loss (~0.4 log CFU/mL) was previously  
279 observed for the encapsulation of *B. breve* into calcium alginate beads (Cook and others 2011) and was  
280 related to dissolution of the capsules rather than the encapsulation itself.

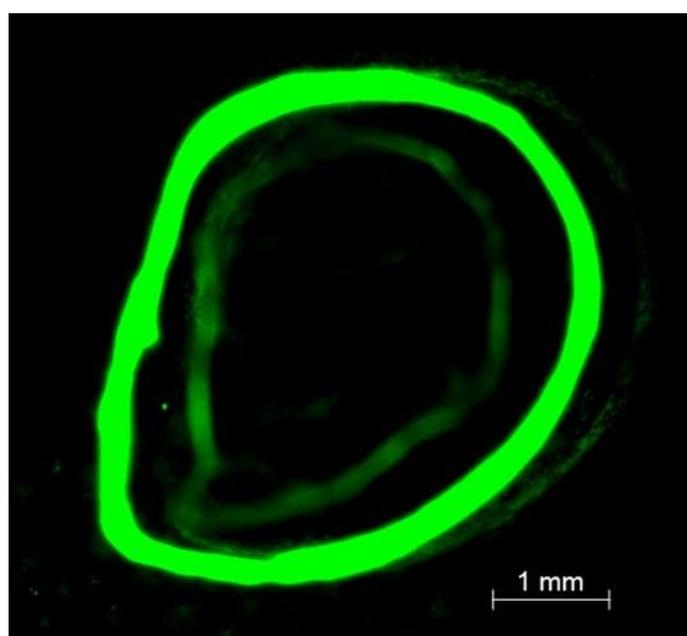
281 It is well known that chitosan has antimicrobial properties (Rabea and others 2003) and its direct  
282 contact with live bacteria could potentially result in reduction of their viability. In order to establish  
283 how deeply chitosan macromolecules can penetrate into calcium pectinate gel, experiments were  
284 performed with FITC-chitosan using fluorescent microscopy. Fig.4 shows a fluorescent image of  
285 calcium pectinate bead coated with FITC chitosan. The bright green band responsible for the  
286 fluorescence of FITC-chitosan indicates that the depth of penetration of chitosan macromolecules into  
287 calcium pectinate gel within 10 min exposure was around  $0.236 \pm 0.061$  mm. This result indicates that

288 chitosan likely forms a coating on the surface of pectinate gels and does not penetrate deeply into  
 289 calcium pectinate. However, this penetration of chitosan into pectinate gel is greater compared to its  
 290 penetration into calcium alginate gels, reported in our previous publication (Cook and others 2011),  
 291 where around 0.007 mm penetration depth was observed. The difference between permeability of these  
 292 two gel materials presents some interest for further studies.



293 Fig.3. Viability of *Lactobacillus casei* during the encapsulation process: a) free cells, b) cells  
 294 encapsulated into 3% calcium pectinate, c) cells encapsulated into 3% calcium pectinate with chitosan  
 295 coating. Amidated pectin was used in these experiments (n=5).  $p$  values denoted by \* ( $p < 0.05$ ),  
 296 signifies statistical difference when compared to free cells.  
 297

298  
 299



300

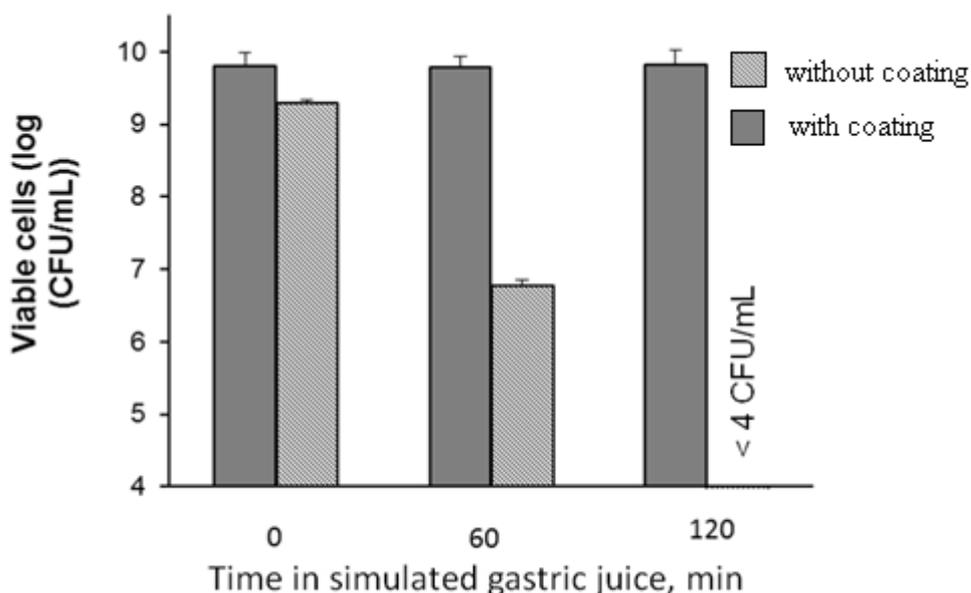
301  
302  
303  
304  
305  
306  
307  
308  
309  
310  
311  
312  
313  
314  
315  
316  
317  
318  
319  
320  
321  
322  
323  
324  
325  
326  
327

Fig.4. Fluorescent microscopy image showing penetration of FITC-chitosan (green) into calcium pectinate capsule.

### 3.4 Viability of encapsulated *Lactobacillus casei* in SGJ (pH 2.0)

Previous studies (Gebara and others 2013; Chotiko and others, 2016) indicated the usefulness of pectin-based materials for encapsulation of various probiotics and highlighted the need for using protective coatings to improve bacteria viability.

Calcium pectinate beads prepared from 3% amidated pectin without and with chitosan coating were evaluated under simulated gastric conditions (2 hours at pH 2.0) to study the effect of encapsulation on the protection to probiotics from the low pH in the stomach. After 120 min of exposure to acidic pH, coated capsules showed very high viability of bacteria without any significant ( $p>0.05$ ) drop in the levels of live cells for up to 120 min. The viability of cells residing within the uncoated beads dropped to less than 7 logs CFU/mL within 60 mins and then to less than 4 logs CFU/mL within 120 mins. Crucially, after 2 hours in simulated gastric conditions the coated capsules showed no loss in cell viability, resulting in a cell recovery of 9.6 logs CFU/mL, which proves that a pectin chitosan coated system effectively protects the cells in a very acidic environment. This also suggests that chitosan coating alone is responsible for total acid protection. The protective effect observed due to chitosan, a basic polysaccharide, is likely to be because of the ability of this polysaccharide to neutralize  $H^+$  ions penetrating into the beads, i.e. chitosan coat acts as a buffering layer preventing acid ingress (Cook et al. 2013). Moreover, it was reported by us previously (Cook et al, 2011) that chitosan-coated alginate beads offered weaker protection compared to chitosan coated pectinate. However, to better understand the protection mechanism, the interaction between pectin and chitosan has to be studied further.



328

329 Fig.5. Viability of encapsulated *Lactobacillus casei* cells in calcium pectinate beads with and without  
 330 chitosan coating. Calcium pectinate beads were prepared using 3% amidated pectin.

331

332

### 333 3.5 Release of encapsulated bacteria in simulated intestinal juice (SIJ)

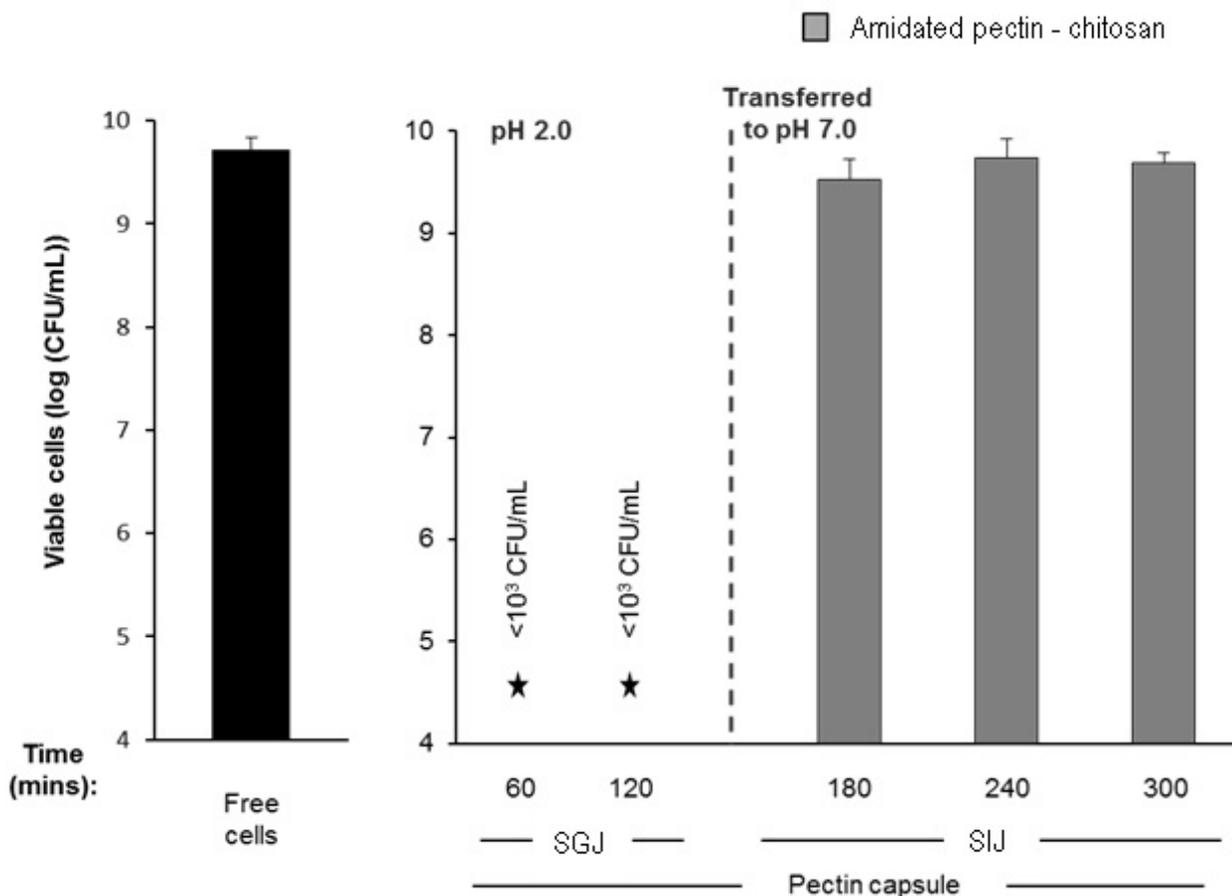
334

335 In order to be effective as enteric delivery system, the capsules must preserve the viability of the  
 336 probiotic cells during their transit through the stomach and deliver these cells alive and in high  
 337 numbers in the intestine (Suita-Cruz & Goulet, 2001).

338 As the produced pectin-chitosan coated capsules provided good results in the acid challenge test,  
 339 their ability to deliver cells to the intestine was investigated. In this *in vitro* experiment,  
 340 gastrointestinal passage was simulated by exposing the capsules to low pH in the stomach during 2  
 341 hours, followed by exposure to the high pH encountered in the intestine for 3 hours. The main aim of  
 342 this experiment was to understand the release profile of the bacteria in the GI tract. It was observed  
 343 that no cells were recovered during gastric transit, but crucially after 1 hour of transferring to SIJ a  
 344 quick release effect was seen and a complete cell recovery was achieved (Fig.6). Disintegration of the  
 345 pectinate capsules and release of bacteria in SIJ observed in this study is in accordance with the  
 346 literature for similar systems. For example, Gebara et al (2013) have also reported complete  
 347 disintegration of pectin microparticles coated with whey protein at pH 7.0. This disintegration is  
 348 related to the dissociation of pectin-chitosan polyelectrolyte complex coating (Birch et al, 2014) as  
 349 well as calcium pectinate gel (Günter and others 2016) under these pH conditions.

350

351



352

353 Fig.6. Release of *Lactobacillus casei* after exposure to SGJ (2 h) and SIJ (3 h), n=4.

354

355 **4. Conclusion**

356

357 Hydrated calcium pectinate capsules containing live *L. casei* were produced using the extrusion  
 358 technique and were subsequently coated with chitosan. Pectin capsules without chitosan coat were  
 359 found to provide limited protection to *L. casei* in simulated gastric juice. Coating with chitosan  
 360 effectively protected the bacterial cells from the acid in the simulated gastric juice and intestinal juice,  
 361 suggesting that these capsules are suitable for gastrointestinal delivery of viable cells. In the future, the  
 362 evaluation of sensory characteristics of these capsules will be of interest.

363

364 **Acknowledgements**

365 This work was funded by the grant from the Ministry of Education and Science, Republic of  
 366 Kazakhstan under the project "Developing immunostimulating dairy products with encapsulated  
 367 synbiotics" (grant number 0115PK01199). The authors also acknowledge Shakarim State University of  
 368 Semey (Kazakhstan) for providing additional funding to support this project.

369

370

371 **References**

372 Albadran H, Chatzifragkou A, Khutoryanskiy VV, Charalampopoulos D. 2015. Stability of  
373 probiotic *Lactobacillus plantarum* in dry microcapsules under accelerated storage conditions. *Food*  
374 *Research International* 74: 208-16.

375 Anal AK, Singh H. 2007. Recent advances in microencapsulation of probiotics for industrial  
376 applications and targeted delivery. *Trends in Food Science & Technology* 18: 240–51.

377 Belitz HD, Grosch W, Schieberle P. 2004. Carbohydrates. In *Food Chemistry*, 245-339. Berlin,  
378 Germany: Springer.

379 Birch NP, Schiffman JD. 2014. Characterization of Self-Assembled Polyelectrolyte Complex  
380 Nanoparticles Formed from Chitosan and Pectin. *Langmuir* 30: 3441–447.

381 Burgain J, Gaiani C, Linder M, Scher J. 2011. Encapsulation of probiotic living cells: From  
382 laboratory scale to industrial applications. *Journal of Food Engineering* 104: 467-83.

383 Chandramouli V, Kailasapathy K, Peiris P, Jones M. 2004. An improved method of  
384 microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions.  
385 *Journal of Microbiological Methods*, 56: 27-35.

386 Chotiko A, Sathivel S. 2016. Three protective agents for pectin-rice bran capsules for  
387 encapsulating *Lactobacillus plantarum*. *Food Bioscience*, 16: 56–65.

388 Cook M, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV. 2011. Production and Evaluation  
389 of Dry Alginate - Chitosan Microcapsules as an Enteric Delivery Vehicle for Probiotic Bacteria.  
390 *Biomacromolecules*, 12: 2834–840.

391 Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV. 2012. Microencapsulation of  
392 probiotics for gastrointestinal delivery. *Journal of Controlled Release*, 162: 56-67.

393 Cook MT, Saratoon T, Tzortzis G, Edwards AD, Charalampopoulos D, Khutoryanskiy VV.  
394 2013. A CLSM method for the dynamic observation of pH change within polymer matrices for oral  
395 delivery. *Biomacromolecules*, 14: 387–93.

396 Cook MT, Tzortzis G, Khutoryanskiy VV, Charalampopoulos D. 2013. Layer-by-layer coating  
397 of alginate matrices with chitosan-alginate for the improved survival and targeted delivery of probiotic  
398 bacteria after oral administration. *Journal of Materials Chemistry. B*, 1: 52-60.

399 Gebara C, Chaves KS, Ribeiro MCE, Souza FN, Grosso CRF, Gigante ML. 2013. Viability of  
400 *Lactobacillus acidophilus* La5 in pectin–whey protein microparticles during exposure to simulated  
401 gastrointestinal conditions. *Food Research International*, 51: 872–78.

- 402 Gerez CL, Font de Valdez G, Gigante ML, Grosso CR. 2012. Whey protein coating bead  
403 improves the survival of the probiotic *Lactobacillus rhamnosus* CRL 1505 to low pH. *Letters in*  
404 *Applied Microbiology*, 54: 552-56.
- 405 Günter EA, Popeyko OV. 2016. Calcium pectinate gel beads obtained from callus cultures  
406 pectins as promising systems for colon-targeted drug delivery. *Carbohydrate Polymers*, 147: 490–99.
- 407 Hansen LT, Allan-Wojtas PM, Jin YL, Paulson AT. 2002. Survival of Ca-alginate  
408 microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. *Food*  
409 *Microbiology*, 19: 35-45.
- 410 Heidebach T, Först P, Kulozik U. 2012. Microencapsulation of Probiotic Cells for Food  
411 Applications. *Critical Reviews in Food Science and Nutrition*, 52: 291-11.
- 412 Morris G, Kök S, Harding S, Adams G. 2010. Polysaccharide drug delivery systems based on  
413 pectin and chitosan. *Biotechnol Genet Eng Rev*, 27: 257-84.
- 414 Muthukumarasamy P, Holley RA. 2006. Microbiological and sensory quality of dry fermented  
415 sausages containing alginate-microencapsulated *Lactobacillus reuteri*. *International Journal of Food*  
416 *Microbiology*, 111: 164–169.
- 417 Nualkaekul S, Cook MT, Khutoryanskiy VV, Charalampopoulos D. 2013. Influence of  
418 encapsulation and coating materials on the survival of *Lactobacillus plantarum* and *Bifidobacterium*  
419 *longum* in fruit juices. *Food Research International*, 53: 304-11.
- 420 Oakenfull D, Scott A. 1984. Hydrophobic interaction in the gelation of high methoxyl pectins.  
421 *Journal of Food Science*, 49: 1093–98.
- 422 Pliszczak D, Bourgeois S, Bordes C, Valour JP, Mazoyer MA, Orecchioni AM, Nakache E,  
423 Lantéri P. 2011. Improvement of an encapsulation process for the preparation of pro- and prebiotics-  
424 loaded bioadhesive microparticles by using experimental design. *European Journal of Pharmaceutical*  
425 *Sciences*, 44: 83–92.
- 426 Rabea EI, Badawy ME-T, Stevens CV, Smagghe G, Steurbaut W. 2003 Chitosan as  
427 Antimicrobial Agent: Applications and Mode of Action, *Biomacromolecules*, 4: 1457–465.
- 428 Reid G. 2016. Probiotics: definition, scope and mechanisms of action. *Best Practice & Research*  
429 *Clinical Gastroenterology*, 30: 17–25.
- 430 Sabikhi L, Babu R, Thompkinson DK, Kapila S. 2010. Resistance of microencapsulated  
431 *Lactobacillus acidophilus* LA1 to processing treatments and simulated gut conditions. *Food and*  
432 *Bioprocess Technology*, 3: 586-93.
- 433 Sandoval-Castilla O, Lobato-Calleros C, García-Galindo HS, Alvarez-Ramírez J, Vernon-Carter  
434 EJ. 2010. Textural properties of alginate–pectin beads and survivability of entrapped *Lb. casei* in  
435 simulated gastrointestinal conditions and in yoghurt. *Food Research International* 43: 111–17.

436 Shi LE, Li ZH, Li DT, Xu M, Chen HY, Zhang ZL, Tang ZX. 2013. Encapsulation of probiotic  
437 *Lactobacillus bulgaricus* in alginate–milk microspheres and evaluation of the survival in simulated  
438 gastrointestinal conditions. *Journal of Food Engineering*, 117: 99–104.

439 Shori AB. 2017. Microencapsulation Improved Probiotics Survival During Gastric Transit.  
440 HAYATI *Journal of Biosciences* 24: 1-5.

441 Siuta-Cruce P, Goulet J. 2001. Improving probiotic survival rates: microencapsulation  
442 preserves the potency of probiotic microorganisms in food systems. *Food Technology*, 55: 36–42.

443 Soumya B, Suvendu B. 2012. Food Gels: Gelling Process and New Applications, *Critical*  
444 *Reviews in Food Science and Nutrition. Food Gels and Application*, 52: 334-46.

445 Thakur BR, Singh RK, Handa AK, Rao MA. 1997. Chemistry and uses of pectin — A review.  
446 *Critical Reviews in Food Science and Nutrition*, 37: 47-73.

447

448

449

450

451

452

453

454

455

456