

1 **Co-encapsulation of *Lactobacillus plantarum* and bioactive compounds extracted from**  
2 **red beet stem (*Beta vulgaris L.*) by spray dryer**

3  
4 Cassandra de Deus<sup>a</sup>, Carlos Eduardo de Souza Brener<sup>b</sup>, Thaianne Marques da Silva<sup>a</sup>, Simara  
5 Somacal<sup>a</sup>, Leila Queiroz Zepka<sup>a</sup>, Eduardo Jacob Lopes<sup>a</sup>, Cristiane de Bona da Silva<sup>b</sup>, Milene  
6 Teixeira Barcia<sup>a</sup>, Jesus Lozano Sanchez<sup>c</sup>, Cristiano Ragagnin de Menezes<sup>a\*</sup>

7 <sup>a</sup>Departament of Food Science and Tecnology, Federal University of Santa Maria, Rio Grande  
8 do Sul, Brazil

9 <sup>b</sup>Departament of Farmacy, Federal University of Santa Maria, Rio Grande do Sul, Brazil

10 <sup>c</sup>Departamento de Nutrición y Bromatología, Universidad de Granada, Granada Spain.

11  
12  
13 Please send correspondence to

14 Cristiano Ragagnin de Menezes, Professor

15 Departament of Food Science and Tecnology

16 Federal University os Santa Maria

17 Phone: (+55) 055981399387; Fax: (+55) 05532208254

18 E-mail: cristiano.ufsm@gmail.com

19  
20  
21 **Abstract**

22 Probiotic bacteria and bioactive compounds obtained from plant origin stand out as ingredients  
23 with the potential to increase the healthiness of functional foods, as there is currently a recurrent  
24 search for them. Probiotics and bioactive compounds are sensitive to intrinsic and extrinsic  
25 factors in the processing and packaging of the finished product. In this sense, the present study  
26 aims to evaluate the co-encapsulation by *spray dryer* (inlet air temperature 120 °C, air flow 40  
27 L / min, pressure of 0.6 MPa and 1.5 mm nozzle diameter) of probiotic bacteria (*L.plantarum*)  
28 and compounds extracted from red beet stems (betalains) in order to verify the interaction  
29 between both and achieve better viability and resistance of the encapsulated material. When  
30 studying the co-encapsulation of *L.plantarum* and betalains extracted from beet stems, an  
31 unexpected influence was observed with a decrease in probiotic viability in the highest  
32 concentration of extract (100%), on the other hand, the concentration of 50% was the best  
33 enabled and maintained the survival of *L.plantarum* in conditions of 25 °C (63.06%), 8 °C  
34 (88.80%) and -18 °C (89.28%). The viability of the betalains and the probiotic was better  
35 preserved in storage at 8 and -18 °C, where the encapsulated stability for 120 days was  
36 successfully achieved. Thus, the polyfunctional formulation developed in this study proved to  
37 be promising, as it expands the possibilities of application and development of new foods.

38 Keywords: probiotic, betalains, bioactive, residue, beet stem, spray dryer.

39

## 40 **1 Introduction**

41

42 The growing demand for quality foods that provide not only nutrition, but also health,  
43 has gained prominence. This directly reflects in the growing and progressive evolution in food  
44 science and nutrition research (Manzoor et al., 2021). The consumer profile of today's post-

45 pandemic is highlighted by the search for a healthy and balanced diet through the acquisition  
46 of new products, which present in their composition ingredients that bring benefits to health.

47         It is already reported that bioactive compounds from natural pigments are trends and if  
48 consumed in a balanced diet can promote health benefits (Cunningham et al., 2021). Similarly,  
49 the theme gut is latent, having as main representatives the probiotic bacteria, which are directly  
50 linked to the balance of the intestinal microbiota , as well as, it is recurrent and strong the appeal  
51 of the narrative, the relationship of the gut with the brain (gut-brain), where it is believed that a  
52 balanced microbiota promotes benefits not only to the gut, but there is also a reflection of this  
53 good functioning on the entire human system (Snigdha et al., 2021). Probiotics are defined by  
54 regulatory bodies as, live microorganisms that when ingested in adequate amounts and with  
55 frequency, have the potential to adhere to the intestinal epithelium in order to promote health  
56 and well-being, and other related secondary benefits (FAO / WHO, 2002; Koirala & Anal,  
57 2021). Probiotics are already known to produce biotherapeutic compounds that exert various  
58 health effects, some *Lactobacillus spp.* strains, such as *Lactobacillus plantarum* help in the  
59 regulation of intestinal microbiota, antioxidant action and antimicrobial against pathogenic  
60 bacteria, regulation of intestinal pH, as well as restoring the microbiota after cases of diarrhea  
61 (Zendeboodi et al., 2020; Echegaray et al., 2023). However, the studies of biologically active  
62 molecules present mainly in plants are contemporary, and have been highlighted in the  
63 development of new products aimed at health and nutrition (Abuga et al., 2021; Pires et al.,  
64 2021). A strong current representative of this class of bioactive molecules are betalains, which  
65 stand out against other compounds because they are relatively more stable to pH changes, have  
66 a unique solubility and high antioxidant activity that promotes health benefits, in addition to a  
67 potential anti-inflammatory and antitumor activities (Spiegel et al., 2021; Martínez-Rodríguez  
68 et al., 2022). Betalains are found in abundance in the beet root, however a challenge is the reuse  
69 of the less noble parts of this tuber, such as the stems and leaves that are usually rejected, yet

70 are matrices as nutritionally and biologically valuable as the beet root (Rodriguez-Amaya,  
71 2019; Coman et al., 2020; Sadowska-Bartosz & Bartosz, 2021a).

72 Both highlighted compounds, suffer from intrinsic (enzymes) and extrinsic (pH,  
73 oxygen, light, temperature) factors when added without some protection in food processing,  
74 moreover, they are negatively affected during human digestion, reducing or completely losing  
75 their efficiency, therefore protecting the efficiency of compounds of interest is a major  
76 challenge that needs to be addressed during the development of new functional food products (  
77 Rezaei et al., 2022, Sawicki et al., 2016, Tossi et al., 2021; Chuah & Mao, 2020). In this sense,  
78 there are already new technologies aimed at reducing this problem (Raddatz & Menezes, 2021).  
79 Among them, microencapsulation by spray dryer is already known and presents a promising  
80 solution in the food area, where it provides a coating of solid or liquid particles inside, with the  
81 premise of targeted delivery of the coated compounds in the exact fraction of the intestine,  
82 where their action will occur, thus resisting the pH changes during digestion (Kuley et al., 2021;  
83 Sharifi et al., 2021a), with the main advantage of obtaining powdered microcapsules, which  
84 facilitate the application and maintenance of the stability of the compounds (Sabarez, 2021).  
85 Therefore, microencapsulation enables the broad exploitation of different compounds inside.  
86 Preserving the effectiveness of probiotic bacteria and bioactive compounds such as betalains is  
87 a recurrent challenge that needs to be addressed during the development of new functional food  
88 products (Vivek et al., 2020, Shaaruddin et al., 2017).

89 Therefore, the present study aimed to co-encapsulate by spray drying atomization  
90 technique probiotic bacteria (*L. plantarum*) with the aqueous extract of betalains obtained from  
91 red beet stem, in order to investigate whether there is a positive influence on the viability,  
92 stability and encapsulation efficiency of both compounds.

93

## 94 2 Materials and Methods

95

### 96 2.1 Materials and Methods

97 The beets *Beta vulgaris* L. (Boron cultivar) were harvested 60 days after planting in a  
98 rural property located in Júlio de Castilho, Rio Grande do Sul - Brazil (-29.153880, -53.643870)  
99 and stored at 8°C until the moment of extraction. Maltodextrin (Ingredion, São Paulo, Brazil),  
100 gum Arabic (CNI, São Paulo, Brazil), trehalose (Hayashibara, São Paulo, Brazil), Twen 80  
101 (Synth, São Paulo, Brazil), glycerol (Enerquímica, Paraná, Brazil) and probiotic culture of  
102 *Lactobacillus Plantarum* LP-01 (*L. plantarum*) obtained by Probiotal (Coana Probiotic  
103 Solution, Santa Catarina, Brazil) were used to develop the microcapsules.

104

### 105 2.2 Preparation of the inoculum

106 The lyophilized strain of *L. plantarum* was activated prior to the microencapsulation  
107 process by adding 1 g of the strain in 100 mL of MRS broth (Merck, Germany) and incubated  
108 for 16 hours at 37°C. The bacteria were harvested in the exponential growth phase by  
109 centrifuged at 2470 x g for 15 minutes at 4°C, and the probiotic mass was washed with NaCl  
110 solution (0.85%) and collected for later addition to the process. The initial concentration of this  
111 activated probiotic culture was approximately ~ 10 log CFU g<sup>-1</sup> (Etchepare et al., 2016).

### 112 2.3 Preparation of betalain extract

113 The beet bundles (10 kg) were properly sanitized (2.5% Cl) and the stems were  
114 separated, then they were frozen for 24h at -18°C, after, freeze-dried for 24h, and then the dried  
115 stems were ground in a knife mill. The extract was obtained following the methodology of

116 OMAE et al. (2017) with some changes described in the sequence: the extract was prepared  
117 with distilled water at 60 °C in a ratio of 1:40 (m/v), followed by vortex agitation for 10 seconds  
118 and centrifuged at 4320 x g, 10 °C for 10 min, the supernatant was collected and filtered under  
119 vacuum on qualitative filter paper (80 g/m<sup>2</sup>).

120

## 121 **2.4 Microcapsule production by spray dryer**

122 The production of the microcapsules was performed following the methodology  
123 proposed previously by Nunes et al. (2017a), with modifications listed in Table 1.

124 The microencapsulation process was performed in a laboratory spray dryer (MSD 1.0  
125 Labmaq, São Paulo, Brazil) with an inlet operating temperature of 120°C and an outlet  
126 temperature of 85°C ± 5°C. The different feed solutions, kept under agitation, were introduced  
127 into the drying system by means of a peristaltic pump with feed rate of 0.47 L / h, drying air  
128 flow rate of 40 L / min, air pressure of 0.6 MPa and a 1.5 mm diameter atomizing nozzle. The  
129 microparticles were collected and transferred to sterile vials and stored.

130

## 131 **2.5 Morphology and average size of microcapsules**

132 The morphology of the dried microcapsules was observed under a scanning electron  
133 microscope (SEM). The average particle size distribution was measured using Mastersizer 3000  
134 laser diffraction equipment (Malvern, Germany), using water as dispersion means.

135

## 136 **2.6 Encapsulation Efficiency (EE%)**

137 Encapsulation efficiency (EE%) was performed for probiotics and the bioactive  
138 compound (betalains) following the methodology suggested by Petraitytė and Sipailienė  
139 (2019), where an equation (Equation 1) was developed that represents the survival rate of  
140 microorganisms and the amount of betalains (EE%) during the microencapsulation process.

141

142 **Equation 1** -  $EE\% = (N/ N_0) \times 100$

143

144 Where N is the number of viable cells (log CFU g<sup>-1</sup>) or betalains (mg / L) released from the  
145 microcapsules, and N<sub>0</sub> is the number of viable cells (log CFU g<sup>-1</sup>) or betalains (mg / L) in the  
146 pre-encapsulation concentrate.

147

## 148 **2.7 Assessing the viability of *L. plantarum* and betalains on storage**

149 In order to determine the viability of *L. plantarum* and the stability of the betalainas  
150 extract both free and microencapsulated, analyses were performed every 15 days, with the  
151 samples stored in sterile vials under the following conditions: 25 ± 1 °C, 8 ± 1 °C and -18 ± 1  
152 °C, for a period of 120 days, according to (Oliveira et al., 2007).

## 153 **2.8 Probiotic viability of *L. plantarum***

154 The quantification of bacteria from the unencapsulated microcapsules was performed  
155 according to the method proposed by Sheu, Marshall and Heymann (1993) with modifications.  
156 The unencapsulation procedure consisted in weighing 0.1 g of sample, adding 9.9 mL of sterile  
157 phosphate buffer solution (pH 7.5) and homogenizing for 10 min in a magnetic stirrer. From  
158 this first dilution, serial decimal dilutions will be made in 0.1% peptone water.

159 For the *L. plantarum* count, 1.0 ml aliquots of the chosen dilutions will be transferred  
160 in triplicates to petri dishes. Over each sample, the MRS Agar culture medium with overlay  
161 will be added. Confirmation of typical colonies will be performed on an electronic colony  
162 counter (LOGEN - LS6000) after incubation for 72 hours under anaerobiosis at 37 °C.

163

## 164 **2.9 Approximate photometric quantification of free and microencapsulated total betalains**

165 The betalain concentration was determined as a function of betanin concentration (mg /  
166 L of extract). For the quantification of betalains inside the microcapsules, followed the  
167 unencapsulation described in section 2.8, then centrifuged the sample at 12000 x g for 5 min at  
168  $10 \pm 1$  °C, separating the residues of the microcapsule, from the extract, obtaining a clear sample  
169 for photometric analysis. The quantification of total betalains followed the methodology  
170 suggested by Herbach; Stintzing; Carle (2004), where the sample (pure extract and encapsulation  
171 free betalains) was diluted with the extracting solvent (water) and quantified in a UV/ VIS  
172 spectrophotometer (Agilent 8453 UV-visible spectrophotometer): Total betalains [mg / L] =  
173  $[(A \times DF \times MW \times 100) / E \times L]$ , where A is the maximum absorption at 536 nm, corrected by  
174 absorption at 600 nm, MW represents the molar mass of betanin (550 g mol<sup>-1</sup>), DF is the  
175 dilution factor, E is the molar absorption (6000 L mol<sup>-1</sup> cm<sup>-1</sup>) and L is the optical path length  
176 (cm).

177

## 178 **2.10 Resistance to heat treatment**

179 Resistance to heat treatment was evaluated as proposed by Zhang, Lin, and Zhong  
180 (2015) with some modifications. For this, 0.1 g of microcapsules and 1 mL of free culture were  
181 transferred to test tubes containing 9 mL of sterile peptone water (0.1%). The contents were

182 then subjected to different thermal conditions, being  $72 \pm 1$  °C for 15 sec and  $63 \pm 1$  °C for 30  
183 min, simulating fast and slow pasteurization respectively. After the pasteurization process, the  
184 tubes were slightly cooled by immersion in ice for 15 min. Finally, aliquots were collected and  
185 cell viability was determined following 2.8.

186

## 187 **2.11 Evaluation of survival of free and microencapsulated *L. plantarum* in simulated** 188 **gastrointestinal conditions**

189 To evaluate the survival of probiotics against the gastrointestinal system, an in vitro  
190 simulation of these conditions was performed following the method proposed by  
191 (MADUREIRA et al., 2011) with modifications. The viability of the bacteria was evaluated  
192 (esophagus/stomach, duodenum, and ileum). During the procedure, aliquots will be taken after  
193 0 min, 90 min (esophagus/stomach), 110 min (duodenum), and 200 min (ileum), analyzing the  
194 survival of free and microencapsulated *L. plantarum* by enumeration on MRS agar according  
195 to item 2.8.

196 In the step where the esophagus/stomach phase was simulated, 0.1g of microcapsules  
197 were weighed followed by homogenization with  $25 \text{ mg mL}^{-1}$  pepsin (Sigma, Germany) in 0.1M  
198 HCl at a concentration of 0.05 mL for 90 min, where 300µl of fractionated enzyme was added  
199 equally in six moments (10, 20, 30, 50, 70 and 90 minutes), and the pH adjusted to 2.0 with 1M  
200 HCl. In the step where the duodenum was simulated, a solution containing 2g pancreatin  
201 (Sigma) and  $12 \text{ g L}^{-1}$  bovine bile salts (Sigma) in 0.1 M  $\text{NaHCO}_3$  at pH 5.0 was used at a  
202 concentration of 0.25 mL, where 125µl of each enzyme in the sample were added. Finally, in  
203 the step where the ileum was simulated, the pH was adjusted to 6.5 using 0.1 M  $\text{NaHCO}_3$ . The  
204 solutions were prepared at the time of analysis and sterilized by membrane filtration (0.20 µm  
205 pore size) (Millipore, Billerica, MA, USA). The analysis was performed in a refrigerated

206 incubator with agitation (TE-421, Tecnal, Piracicaba, SP, Brazil) at 37°C simulating body  
207 temperature and agitation conditions to simulate the peristaltic movements of the digestive tract.

208

## 209 **2.12 Statistical analysis**

210 The analyses were performed in triplicate and the significant differences between the  
211 means for the values obtained were submitted to analysis of variance (ANOVA) and Tukey's  
212 test with the aid of the STATISTICA® version 10.0 program at a 5% significance level.

213

## 214 **3. Results and Discussion**

215

### 216 **3.1 Characterization of microcapsules obtained by spray dryer**

217 The characterization of microcapsules obtained by spray drying were described in table  
218 2, where the viability, average particle size (Span <2,5) and the EE of probiotics and betalains  
219 were evaluated. It is observed that probably the amount of extract replaced in the formulations  
220 and the used solids concentration of 12% interfered significantly in the microcapsule size and  
221 EE of both treatments (Antigo et al., 2020), in addition, it is verified a possible antimicrobial  
222 action (Hu et al., 2020, Lages et al., 2021) attributed to betalains, being atypical results to the  
223 findings by Machado Vasconcelos et al., (2021) and Jouki et al., (2021). However a recent study  
224 by Pupa et al., (2021a) reveals similar results to those described in Table 3, where by  
225 encapsulating *L. plantarum* by spray dryer achieved ~ 75% encapsulation efficiency and  
226 average particle size  $\pm 12.88 \mu\text{m}$ .

227           The morphology of the microcapsules was elucidated by MEV images (Figure 1) with  
228 magnification ranging from 500 to 8000 x. In general, for A, B, E and F presented rounded  
229 structures with apparent roughness and agglomerated, and for C and D disuniform  
230 microcapsules with no defined structure, in addition to agglomeration and irregular surfaces  
231 (Sharifi et al., 2021b). However, no cracking was observed on the surfaces of both treatments,  
232 being a positive aspect, as it makes it impossible for oxygen to enter that can degrade both  
233 compounds (Costa et al., 2023). The conditions used in the atomization process were adequate  
234 to obtain microcapsules proportional to the findings of Nunes et al. (2017b) and Neuenfeldt et  
235 al. (2022) even when applying higher temperatures and flows. However, higher temperatures  
236 are often used when microencapsulating betalain pigments, as reported in studies by Delia et  
237 al. (2019) and Yang et al. (2021) where the inlet air temperatures were 140 °C and 170 °C  
238 respectively, conditions that are unlikely to be applied when it comes to the development of  
239 symbiotic microcapsules with probiotics.

240

### 241 **3.2 Evaluation of the stability of free and microencapsulated *L. plantarum* and betalainas** 242 **in storage**

243           The viability of the microencapsulated probiotic in T4 in the storage condition at 25 °C  
244 depicted in Figure 2 (A) was higher than treatments T3 and T5, being lower only than the free  
245 cell control (T1) that remained high in both storage conditions tested, resulting at the end of  
246 120 days, survival of 62.19% in T1 and 63.09% for T5, other treatments did not resist for 120  
247 days of storage. It is assumed that the 50% amount of extract added in the formulation may  
248 have protected the probiotic from the process of exposure to heat during the spray dryer, besides  
249 being a propitious amount where the antimicrobial effects of betalains have low activity against  
250 the probiotics. In the storage conditions at 8 °C and -18 °C, treatments T1, T3, T4 and T5

251 throughout the 120 days, remained similar, showing viable with approximately ~ 6 log CFU g<sup>-1</sup>,  
252 <sup>1</sup>, with survival at end of 120 days of 60.41%, 89.74, 85.12% and 89.28% respectively. Thus,  
253 this represents a positive result against the minimum limit required for a beneficial action in the  
254 human intestine (Yoha et al., 2021).

255 The present finding proved to be superior to the literature reports, and the storage  
256 conditions simulated in this work are already reproduced and are promising references to enable  
257 the survival of probiotics as reported by (Coghetto et al., 2016) where they encapsulated *L.*  
258 *plantarum* by electrospray with refrigerated storage at 4 °C for 21 days, and (Mahmoud et al.,  
259 2020) who encapsulated *L. plantarum* by extrusion and storage under refrigeration at 6 °C and  
260 freezing at -18 °C for one to three months. However, the results discovered by the authors are  
261 similar or lower than the findings in this study for viability of *L. plantarum* stored for 120 days  
262 under three simulated conditions. However, both authors come into agreement, stating that  
263 refrigeration and freezing temperature is the best condition to maintain survival of *L. plantarum*,  
264 thus corroborating with the findings. Similarly, Pupa et al., (2021b) reported that spray dryer  
265 encapsulation of probiotic bacteria proved to be more efficient compared to techniques such as  
266 extrusion and emulsification, identifying in the dry samples lower loss of viability during  
267 storage, thus attributed this positive result to the low water activity of the microcapsules.

268 In the same way that the protection of probiotics was achieved under the circumstances  
269 studied, the beet stem extract was also protected and remained stable in both treatments that  
270 underwent encapsulation (T4 and T5), as can be seen in Figure 2 in D, E and F, where only the  
271 results of the treatments that contained the reddish pigment in their composition were expressed.  
272 Over time, T5 was the treatment that varied the least at 25 °C for the extract, but T4, as it  
273 retained a higher concentration of betalains, remained with high concentrations, just as T5, with  
274 50% extract, remained with significant amounts of total betalains estimated in both conditions  
275 studied. The microencapsulation of betalains from different sources and techniques has been

276 studied, as reported by Pagano et al., (2018) where he studied the A/O/A type emulsion, thus  
277 verifying the efficiency in intestinal digestion in vitro. In parallel, Hidalgo et al., (2018)  
278 preserved the bioactive and pigmented compounds obtained from beet pomace by freeze-drying  
279 and successfully applied them in cookies. In addition, nanoencapsulation was also studied in  
280 2018 by Amjadi et al. where they developed liposomal nanocarriers with betalains that were  
281 successfully applied in gummy candies. Thus, it is evident that the microencapsulation of  
282 betalain pigments obtained from beet stem has shown promise as it promoted protection and  
283 thus will enable its application in food systems. In contrast, the rapid loss of stability of the  
284 extract containing betalain pigments in free form (T2), stored at 25 °C and 8 °C shown in Figure  
285 2 (D and E) was predictable, because it is already known in the literature that betalains are  
286 unstable pigments and sensitive to degradation when stored without previous treatments  
287 (Skalicky et al., 2020, Silva et al., 2020). In recent studies, it has been proven that the instability  
288 of betalain in its free form is of concern, having as a characteristic a first-order degradation  
289 kinetics (Laqui-Vilca et al., 2018; Fernando et al., 2021), thus the more concentrated the  
290 compound is the faster its degradation will be, with this, it becomes unfeasible the application  
291 in free form of this natural pigment in food systems (Sravan Kumar et al., 2020).

292           Although the storage temperatures at 25 °C and 8 °C had significant variations for both  
293 compounds (probiotic and betalains), the microencapsulated or free-encapsulated treatments  
294 stored under freezing condition (-18 °C) proved to have a high efficiency in preserving the  
295 viability and stability of both compounds of interest studied, corroborating with the findings of  
296 Kayın et al., (2019) and Bianchi et al., (2021a).

297

298 **3.4 Evaluation of the resistance of microencapsulated and free *L. plantarum* and betalains**  
299 **to heat treatment**

300           The heat resistance (Figure 3) of the microencapsulated compounds was tested, in order  
301 to verify their application in foods that undergo previous processing as performed. Thus, it was  
302 observed that encapsulated bacteria exhibited a lower number of viable cells than free bacteria.  
303 This event can be explained in parts, as the encapsulated treatments underwent thermal stress  
304 from the encapsulation process, temperatures that did not reach the free cells. discovered by  
305 Marins et al. (2022), when evaluating the resistance of *L. plantarum* when applied to  
306 hamburgers baked for 6 minutes at 72 °C, thus confirming a greater probiotic survival.  
307 However, it should be noted that T1 did not suffer with previous heating as treatments T3, T4  
308 and T5, which underwent heating of 120 °C in the encapsulation process, and subsequent heat  
309 treatment, thus raising its vulnerability during the thermal process studied. However, among  
310 the microencapsulated treatments, T5 was the most efficient in preserving probiotic viability at  
311 73 °C. On the other hand, compared to T4, the standard T3 made the probiotics unviable to a  
312 greater extent. On the other hand, heating at 63 °C for 30 minutes proved to be more efficient  
313 for T3 and T5 than for T4. It is believed that the high concentration of extract present in T4  
314 may have caused this unexpected result, since betalains have an already elucidated  
315 antimicrobial activity that may be intervening in the viability of the probiotic (Chhikara et al.,  
316 2019, Sadowska-Bartosz & Bartosz, 2021b).

317           The stability of the heat treatment of both free and encapsulated extracts was more  
318 promising in the simulation of 63 °C for T2 and T4, except for T5, which obtained better  
319 stability at 72 °C possibly because it is a faster process. Similar results to the findings were  
320 identified by Dias et al., (2020), Kaur et al., (2021) and Bianchi et al., (2021b), where they  
321 evaluated the stability in pasteurization process of betalains in cooked ham, pure beet and juices  
322 (apple and beet) respectively. Also, Liu et al., (2018) reports in his findings that *Lactobacillus*  
323 *rhamnosus* survive at high temperatures in the spray dryer drying process, but suffer a  
324 considerable decline when exposed to heat treatment. Another relevant factor accused by the

325 author, is in the low protection provided by trehalose, which until then is considered a good  
326 thermoprotectant for the microencapsulation of probiotics (Nunes et al. (2017c), not being in  
327 its entirety, efficient in protecting *L. plantarum*.

328 It is assumed that the results obtained for this test are directly related to the concentration  
329 of the extract inside the microcapsule, also, it is observed that the spray dryer  
330 microencapsulation process at a concentration of ~ 13% solids, was not able to completely  
331 protect the probiotics and in parts the betalains. Even so, the T5 treatment showed superiority  
332 in protecting both overall.

333

### 334 **3.5 Evaluation of the resistance of microencapsulated and free *L. plantarum* in simulated** 335 **gastrointestinal conditions**

336 The result of the viable cell counts of free *L. plantarum* and in the different  
337 microencapsulated treatments with and without extract related to the simulated digestive system  
338 conditions are depicted in Figure 4.

339 When starting the simulation, treatment T1 showed count below 4 log CFU g<sup>-1</sup>, in  
340 contrast, all microencapsulated T3, T4 and T5 showed no count in the simulated stomach stage,  
341 which reached pH 2 after 90 minutes. This result represents a good ability of the microcapsule  
342 structure to withstand passage in adverse environments such as low stomach pH. Teo et al.,  
343 2021 and Misra et al., 2021 points out that wall materials such as gum arabic, trehalose and  
344 maltodextrin are ideal for the development of microcapsules produced by spray dryer as they  
345 exert a thermo protection to the compounds of interest, a good yield and encapsulation  
346 efficiency.

347 After 90 minutes in the simulated stomach, the simulation of the duodenum phase  
348 began, so the samples remained at pH 5 for 20 minutes, where T1 showed a count of 4.4 log  
349 CFU g<sup>-1</sup>. Also in this phase, treatments T3, T4, and T5 showed characteristic of partial de-  
350 encapsulation, and counts below 4 log CFU g<sup>-1</sup> were depicted. Finally, in the ileum phase was  
351 reproduced at pH 6.5, where the free cell signaled a slight log decrease ( $3.58 \pm 0.06$ ) compared  
352 to the encapsulated treatments T3 ( $3.67 \pm 0.11$ ), T4 ( $3.81 \pm 0.03$ ) and T5 ( $3.71 \pm 0.08$ ).

353 Paula et al., (2019) describes a reduction in viability of 80.4% and 25.0% for the  
354 microencapsulated and free probiotic respectively after in vitro simulation of gastrointestinal  
355 conditions. However, it is believed that depending on the applied method, encapsulation  
356 conditions, probiotic strains used, composition and concentration of the wall material, different  
357 results are obtained, as reported by Homayouni-Rad et al. (2021). In contrast, Emser et al.,  
358 (2017), found a slight superiority of *L. plantarum* when exposed to a rapid simulation of passage  
359 through the digestive system. Despite the average amount of 3.73 log CFU g<sup>-1</sup> of the  
360 encapsulated treatments delivered at the end of the digestive system (ileum), it is believed that  
361 there was a controlled release of the microencapsulated probiotics during the simulation, thus  
362 demonstrating a positive result in the protection of probiotics and gradual delivery of probiotics.  
363 A controlled release of the microencapsulated probiotics is observed in figure 4, as the  
364 microcapsules partially ruptured in the duodenum phase and finalizes the rupture in the ileum  
365 phase. However, future investigation of the viability of both compounds in in vivo digestive  
366 systems is relevant (Westfall et al., 2021).

367

#### 368 **4 Conclusion**

369 The co-encapsulation of *L. plantarum* and betalains extracted from red beet stem was  
370 successfully achieved. It was found that the characterization parameters such as encapsulation

371 efficiency, morphology and average diameter of the microcapsules obtained, corroborated with  
372 the other studies already described in the literature, including the encapsulation of other extracts  
373 by spray dryer. In the evaluation of the viability of both microencapsulated compounds in the  
374 period of 120 days, the results were promising, being suitable for application in foods that do  
375 not require heat treatment before packaging, suggesting the application of the developed  
376 encapsulates in edible ice creams such as ice cream, non-pasteurized beverages such as  
377 kombucha and cold-pressed juices, besides the application in butters and other refrigerated or  
378 frozen foods. Regarding the stability of the microencapsulated betalaínas extract, success was  
379 obtained in the protection of this pigment with satisfactory results, because as observed in the  
380 storage at temperatures of 25 °C and 8 °C of the extract without the protection of the  
381 microcapsule, the stability was lost in 15 days, extremely important result with regard to an  
382 alternative for reuse and which will have an impact on the reduction of the generation of agro-  
383 industrial waste, as well as the application of a material with great bioactive potential to be  
384 explored and inserted in human food, since betalains are associated health promotion with  
385 antioxidant, anti-inflammatory and antitumor potential. In the simulation of the digestive  
386 system, a controlled and gradual release of probiotics was observed in the duodenum and ileum  
387 phases, a result that emphasizes the arrival and potential performance of probiotics in the  
388 intestine, promoting benefits to health of the individual, mainly in the rebalancing of the  
389 intestinal microbiota, antioxidant, antimicrobial action against pathogens, in addition to helping  
390 to reinforce natural defenses. Thus, for the first time, success was obtained in the co-  
391 encapsulation by spray dryer of *L. plantarum* and betalaínas, making possible the application  
392 of this technology in the protection of both compounds, proposing the application of this  
393 polyfunctional formulation in foods that do not undergo thermal treatment before packaging. In  
394 view of the above, it is noted that the use of beet stem, which is currently an agro-industrial  
395 residue, can be applied to new technologies in the preparation of superfoods in order to reduce

396 environmental impacts and add value to this nutrient-rich vegetable matrix that is still little  
397 explored.

398

## 399 **5 References**

400

401 Abuga, I., Sulaiman, S. F., Wahab, R. A., Ooi, K. L., & Rasad, M. S. B. A. (2021).

402 Phytochemical constituents and antibacterial activities of 45 Malay traditional medicinal

403 plants. *Journal of Herbal Medicine*, 100496. <https://doi.org/10.1016/J.HERMED.2021.100496>

404 Amjadi, S., Ghorbani, M., Hamishehkar, H., & Roufegarinejad, L. (2018). Improvement in

405 the stability of betanin by liposomal nanocarriers: Its application in gummy candy as a food

406 model. *Food Chemistry*, 256, 156–162. <https://doi.org/10.1016/J.FOODCHEM.2018.02.114>

407 Antigo, J. L. D., Stafussa, A. P., de Cassia Bergamasco, R., & Madrona, G. S. (2020). Chia

408 seed mucilage as a potential encapsulating agent of a natural food dye. *Journal of Food*

409 *Engineering*, 285, 110101. <https://doi.org/10.1016/J.JFOODENG.2020.110101>

410 Bianchi, F., Pünsch, M., & Venir, E. (2021). Effect of Processing and Storage on the Quality

411 of Beetroot and Apple Mixed Juice. *Foods 2021*, Vol. 10, Page 1052, 10(5), 1052.

412 <https://doi.org/10.3390/FOODS10051052>

413 Chhikara, N., Kushwaha, K., Sharma, P., Gat, Y., & Panghal, A. (2019). Bioactive

414 compounds of beetroot and utilization in food processing industry: A critical review. In *Food*

415 *Chemistry* (Vol. 272, pp. 192–200). Elsevier Ltd.

416 <https://doi.org/10.1016/j.foodchem.2018.08.022>

417 Chuah, L. O., & Mao, Y. (2020). Stability assessment and improvement of a *Lactobacillus*  
418 *plantarum* mutant with low post-fermentation acidification characteristics. *Journal of Dairy*  
419 *Science*, 103(9), 7898–7907. <https://doi.org/10.3168/JDS.2020-18285>

420 Coghetto, C. C., Brinques, G. B., Siqueira, N. M., Pletsch, J., Soares, R. M. D., & Ayub, M.  
421 A. Z. (2016). Electrospraying microencapsulation of *Lactobacillus plantarum* enhances cell  
422 viability under refrigeration storage and simulated gastric and intestinal fluids. *Journal of*  
423 *Functional Foods*, 24, 316–326. <https://doi.org/10.1016/J.JFF.2016.03.036>

424 Coman, V., Teleky, B. E., Mitrea, L., Martău, G. A., Szabo, K., Călinoiu, L. F., & Vodnar, D.  
425 C. (2020). Bioactive potential of fruit and vegetable wastes. *Advances in Food and Nutrition*  
426 *Research*, 91, 157–225. <https://doi.org/10.1016/BS.AFNR.2019.07.001>

427 Costa, N. de A., Silveira, L. R., Amaral, E. de P., Pereira, G. C., Paula, D. de A., Vieira, É. N.  
428 R., Martins, E. M. F., Stringheta, P. C., Leite Júnior, B. R. de C., & Ramos, A. M. (2023).  
429 Use of maltodextrin, sweet potato flour, pectin and gelatin as wall material for  
430 microencapsulating *Lactiplantibacillus plantarum* by spray drying: Thermal resistance, in  
431 vitro release behavior, storage stability and physicochemical properties. *Food Research*  
432 *International*, 164, 112367. <https://doi.org/10.1016/J.FOODRES.2022.112367>

433 Cunningham, M., Azcarate-Peril, M. A., Barnard, A., Benoit, V., Grimaldi, R., Guyonnet, D.,  
434 Holscher, H. D., Hunter, K., Manurung, S., Obis, D., Petrova, M. I., Steinert, R. E., Swanson,  
435 K. S., van Sinderen, D., Vulevic, J., & Gibson, G. R. (2021). Shaping the Future of Probiotics  
436 and Prebiotics. *Trends in Microbiology*, 29(8), 667–685.  
437 <https://doi.org/10.1016/J.TIM.2021.01.003>

438 Delia, S. C., Chávez, G. M., León-Martínez Frank, M., Araceli, S. G. P., Irais, A. L., &  
439 Franco, A. A. (2019). Spray drying microencapsulation of betalain rich extracts from

440 Escontria chiotilla and Stenocereus queretaroensis fruits using cactus mucilage. *Food*  
441 *Chemistry*, 272, 715–722. <https://doi.org/10.1016/J.FOODCHEM.2018.08.069>

442 Dias, S., Castanheira, E. M. S., Fortes, A. G., Pereira, D. M., Rodrigues, A. R. O., Pereira, R.,  
443 & Gonçalves, M. S. T. (2020). Application of Natural Pigments in Ordinary Cooked Ham.  
444 *Molecules* 2020, Vol. 25, Page 2241, 25(9), 2241.  
445 <https://doi.org/10.3390/MOLECULES25092241>

446 Echegaray, N., Yilmaz, B., Sharma, H., Kumar, M., Pateiro, M., Ozogul, F., & Lorenzo, J. M.  
447 (2023). A novel approach to *Lactiplantibacillus plantarum*: From probiotic properties to the  
448 omics insights. *Microbiological Research*, 268, 127289.

449 Emser, K., Barbosa, J., Teixeira, P., & Bernardo de Morais, A. M. M. (2017). *Lactobacillus*  
450 *plantarum* survival during the osmotic dehydration and storage of probiotic cut apple. *Journal*  
451 *of Functional Foods*, 38, 519–528. <https://doi.org/10.1016/J.JFF.2017.09.021>

452 Etchepare, M. de A., Raddatz, G. C., Cichoski, A. J., Flores, É. M. M., Barin, J. S., Queiroz  
453 Zepka, L., Jacob-Lopes, E., Grosso, C. R. F., & de Menezes, C. R. (2016). Effect of resistant  
454 starch (Hi-maize) on the survival of *Lactobacillus acidophilus* microencapsulated with sodium  
455 alginate. *Journal of Functional Foods*, 21, 321–329. <https://doi.org/10.1016/j.jff.2015.12.025>

456 Etchepare, M. de A., Nunes, G. L., Nicoloso, B. R., Barin, J. S., Moraes Flores, E. M., de  
457 Oliveira Mello, R., & Ragagnin de Menezes, C. (2020). Improvement of the viability of  
458 encapsulated probiotics using whey proteins. *LWT*, 117, 108601.  
459 <https://doi.org/10.1016/J.LWT.2019.108601>

460 FAO/WHO (2002) Guidelines for the Evaluation of Probiotics in Food. Food and Agriculture  
461 Organization of the United Nations/World Health Organization, London,  
462 Ontario.[www.who.int/foodsafety/fs\\_management/en/probiotic\\_guidelines.pdf](http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf)

463 Fernando, G. S. N., Wood, K., Papaioannou, E. H., Marshall, L. J., Sergeeva, N. N., &  
464 Boesch, C. (2021). Application of an Ultrasound-Assisted Extraction Method to Recover  
465 Betalains and Polyphenols from Red Beetroot Waste. *ACS Sustainable Chemistry &*  
466 *Engineering*, 9(26), 8736–8747. <https://doi.org/10.1021/ACSSUSCHEMENG.1C01203>

467 Herbach, K. M., Stintzing, F. C., & Carle, R. (2004). Impact of thermal treatment on color and  
468 pigment pattern of red beet (*Beta vulgaris* L.) preparations. *Journal of Food Science*, 69(6).  
469 <https://doi.org/10.1111/J.1365-2621.2004.TB10994.X>

470 Hidalgo, A., Brandolini, A., Čanadanović-Brunet, J., Četković, G., & Tumbas Šaponjac, V.  
471 (2018). Microencapsulates and extracts from red beetroot pomace modify antioxidant  
472 capacity, heat damage and colour of pseudocereals-enriched einkorn water biscuits. *Food*  
473 *Chemistry*, 268, 40–48. <https://doi.org/10.1016/J.FOODCHEM.2018.06.062>

474 Homayouni-Rad, A., Mortazavian, A. M., Mashkani, M. G., Hajipour, N., & Pourjafar, H.  
475 (2021). Effect of *Alyssum homolocarpum* mucilage and inulin microencapsulation on the  
476 survivability of *Lactobacillus casei* in simulated gastrointestinal and high-temperature  
477 conditions. *Biocatalysis and Agricultural Biotechnology*, 35, 102075.  
478 <https://doi.org/10.1016/J.BCAB.2021.102075>

479 Hu, H., Yao, X., Qin, Y., Yong, H., & Liu, J. (2020). Development of multifunctional food  
480 packaging by incorporating betalains from vegetable amaranth (*Amaranthus tricolor* L.) into  
481 quaternary ammonium chitosan/fish gelatin blend films. *International Journal of Biological*  
482 *Macromolecules*, 159, 675–684. <https://doi.org/10.1016/J.IJBIOMAC.2020.05.103>

483 Jouki, M., Khazaei, N., Rezaei, F., & Taghavian-Saeid, R. (2021). Production of synbiotic  
484 freeze-dried yoghurt powder using microencapsulation and cryopreservation of *L. plantarum*  
485 in alginate-skim milk microcapsules. *International Dairy Journal*, 122, 105133.  
486 <https://doi.org/10.1016/J.IDAIRYJ.2021.105133>

487 Kaur, S., Kaur, N., Aggarwal, P., & Grover, K. (2021). Bioactive compounds, antioxidant  
488 activity, and color retention of beetroot (*Beta vulgaris* L.) powder: Effect of steam blanching  
489 with refrigeration and storage. *Journal of Food Processing and Preservation*, 45(3), e15247.  
490 <https://doi.org/10.1111/JFPP.15247>

491 Kayın, N., Atalay, D., Türken Akçay, T., & Erge, H. S. (2019). Color stability and change in  
492 bioactive compounds of red beet juice concentrate stored at different temperatures. *Journal of*  
493 *Food Science and Technology*, 56(11), 5097–5106. <https://doi.org/10.1007/s13197-019->  
494 03982-5

495 Koirala, S., & Anal, A. K. (2021). Probiotics-based foods and beverages as future foods and  
496 their overall safety and regulatory claims. *Future Foods*, 3, 100013.  
497 <https://doi.org/10.1016/J.FUFO.2021.100013>

498 Kuley, E., Kuscu, M. M., Durmus, M., & Ucar, Y. (2021). Inhibitory activity of Co-  
499 microencapsulation of cell free supernatant from *Lactobacillus plantarum* with propolis  
500 extracts towards fish spoilage bacteria. *LWT*, 146, 111433.  
501 <https://doi.org/10.1016/J.LWT.2021.111433>

502 Lages, L. Z., Radünz, M., Gonçalves, B. T., Silva da Rosa, R., Fouchy, M. V., de Cássia dos  
503 Santos da Conceição, R., Gularte, M. A., Barboza Mendonça, C. R., & Gandra, E. A. (2021).  
504 Microbiological and sensory evaluation of meat sausage using thyme (*Thymus vulgaris*, L.)  
505 essential oil and powdered beet juice (*Beta vulgaris* L., Early Wonder cultivar). *LWT*, 148,  
506 111794. <https://doi.org/10.1016/J.LWT.2021.111794>

507 Laqui-Vilca, C., Aguilar-Tuesta, S., Mamani-Navarro, W., Montañó-Bustamante, J., &  
508 Condezo-Hoyos, L. (2018). Ultrasound-assisted optimal extraction and thermal stability of  
509 betalains from colored quinoa (*Chenopodium quinoa* Willd) hulls. *Industrial Crops and*  
510 *Products*, 111, 606–614. <https://doi.org/10.1016/J.INDCROP.2017.11.034>

511 Liu, B., Fu, N., Woo, M. W., & Chen, X. D. (2018). Heat stability of *Lactobacillus rhamnosus*  
512 GG and its cellular membrane during droplet drying and heat treatment. *Food Research*  
513 *International*, 112, 56–65. <https://doi.org/10.1016/J.FOODRES.2018.06.006>

514 Machado Vasconcelos, L. I., Silva-Buzanello, R. A. da, Kalschne, D. L., Scremin, F. R.,  
515 Stival Bittencourt, P. R., Gaudêncio Dias, J. T., Canan, C., & Corso, M. P. (2021). Functional  
516 fermented sausages incorporated with microencapsulated *Lactobacillus plantarum* BG 112 in  
517 Acrycoat S100. *LWT*, 148, 111596. <https://doi.org/10.1016/J.LWT.2021.111596>

518 Madureira, A. R., Amorim, M., Gomes, A. M., Pintado, M. E., & Malcata, F. X. (2011).  
519 Protective effect of whey cheese matrix on probiotic strains exposed to simulated  
520 gastrointestinal conditions. *Food Research International*, 44(1), 465–470.  
521 <https://doi.org/10.1016/j.foodres.2010.09.010>

522 Mahmoud, M., Abdallah, N. A., El-Shafei, K., Tawfik, N. F., & El-Sayed, H. S. (2020).  
523 Survivability of alginate-microencapsulated *Lactobacillus plantarum* during storage,  
524 simulated food processing and gastrointestinal conditions. *Heliyon*, 6(3), e03541.  
525 <https://doi.org/10.1016/J.HELIYON.2020.E03541>

526 Manzoor, M., Singh, J., Gani, A., & Noor, N. (2021, May 16). Valorization of natural colors  
527 as health-promoting bioactive compounds: Phytochemical profile, extraction techniques, and  
528 pharmacological perspectives - ScienceDirect. *Food Chemistry* 362, 130–141. [https://www-](https://www-sciencedirect.ez47.periodicos.capes.gov.br/science/article/pii/S030881462101147X)  
529 [sciencedirect.ez47.periodicos.capes.gov.br/science/article/pii/S030881462101147X](https://www-sciencedirect.ez47.periodicos.capes.gov.br/science/article/pii/S030881462101147X)

530 Marins, A. R., de Campos, T. A. F., Pereira Batista, A. F., Correa, V. G., Peralta, R. M.,  
531 Graton Mikcha, J. M., Gomes, R. G., & Feihmann, A. C. (2022). Effect of the addition of  
532 encapsulated *Lactiplantibacillus plantarum* Lp-115, *Bifidobacterium animalis* spp. *lactis* Bb-  
533 12, and *Lactobacillus acidophilus* La-5 to cooked burger. *LWT*, 155.  
534 <https://doi.org/10.1016/J.LWT.2021.112946>

535 Martínez-Rodríguez, P., Guerrero-Rubio, M. A., Henarejos-Escudero, P., García-Carmona, F.,  
536 & Gandía-Herrero, F. (2022). Health-promoting potential of betalains in vivo and their  
537 relevance as functional ingredients: A review. *Trends in Food Science and Technology*, *122*,  
538 66–82. <https://doi.org/10.1016/J.TIFS.2022.02.020>

539 Misra, S., Pandey, P., & Mishra, H. N. (2021). Novel approaches for co-encapsulation of  
540 probiotic bacteria with bioactive compounds, their health benefits and functional food product  
541 development: A review. *Trends in Food Science & Technology*, *109*, 340–351.  
542 <https://doi.org/10.1016/J.TIFS.2021.01.039>

543 Neuenfeldt, N. H., Almeida Farias, C. A., de Oliveira Mello, R., Robalo, S. S., Barin, J. S.,  
544 Picolli da Silva, L., Müller, E. I., Moraes Flores, E. M., Barcia, M. T., & Ragagnin de  
545 Menezes, C. (2022). Effects of blueberry extract co-microencapsulation on the survival of  
546 *Lactobacillus rhamnosus*. *LWT*, *155*, 112886. <https://doi.org/10.1016/J.LWT.2021.112886>

547 Nunes, G. L., Etchepare, M. de A., Cichoski, A. J., Zepka, L. Q., Jacob Lopes, E., Barin, J. S.,  
548 Flores, É. M. de M., da Silva, C. de B., & de Menezes, C. R. (2018). Inulin, hi-maize, and  
549 trehalose as thermal protectants for increasing viability of *Lactobacillus acidophilus*  
550 encapsulated by spray drying. *LWT - Food Science and Technology*, *89*(October 2017), 128–  
551 133. <https://doi.org/10.1016/j.lwt.2017.10.032>

552 Oliveira, A. C., Moretti, T. S., Boschini, C., Baliero, J. C. C., Freitas, O., & Favaro-Trindade,  
553 C. S. (2007). Stability of microencapsulated *B. lactis* (BI 01) and *L. acidophilus* (LAC 4) by  
554 complex coacervation followed by spray drying. *Journal of Microencapsulation*, *24*(7), 685–  
555 693. <https://doi.org/10.1080/02652040701532908>

556 Omae, J. M., Goto, P. A., Rodrigues, L. M., dos Santos, S. S., Paraiso, C. M., Madrona, G. S.,  
557 & de Cássia Bergamasco, R. (2017). Beetroot extract encapsulated in inulin: Storage stability

558 and incorporation in sorbet. *Chemical Engineering Transactions*, 57, 1843–1848.  
559 <https://doi.org/10.3303/CET1757308>

560 Ortiz-Basurto, R. I., Rubio-Ibarra, M. E., Ragazzo-Sanchez, J. A., Beristain, C. I., & Jiménez-  
561 Fernández, M. (2017). Microencapsulation of *Eugenia uniflora* L. juice by spray drying using  
562 fructans with different degrees of polymerisation. *Carbohydrate Polymers*, 175, 603–609.  
563 <https://doi.org/10.1016/J.CARBPOL.2017.08.030>

564 Otalora, C. M., Bonifazi, E., Fissore, E. N., Basanta, F., & Gerschenson, L. N. (2020).  
565 Thermal Stability of Betalains in By-Products of the Blanching and Cutting of *Beta vulgaris*  
566 *L. var conditiva*. *Polish Journal of Food and Nutrition Sciences*, 70(1), 15–24.  
567 <http://journal.pan.olsztyn.pl>

568 Pagano, A. P. E., Khalid, N., Kobayashi, I., Nakajima, M., Neves, M. A., & Bastos, E. L.  
569 (2018). Microencapsulation of betanin in monodisperse W/O/W emulsions. *Food Research*  
570 *International*, 109, 489–496. <https://doi.org/10.1016/J.FOODRES.2018.04.053>

571 Paula, D. de A., Martins, E. M. F., Costa, N. de A., de Oliveira, P. M., de Oliveira, E. B., &  
572 Ramos, A. M. (2019). Use of gelatin and gum arabic for microencapsulation of probiotic cells  
573 from *Lactobacillus plantarum* by a dual process combining double emulsification followed by  
574 complex coacervation. *International Journal of Biological Macromolecules*, 133, 722–731.  
575 <https://doi.org/10.1016/J.IJBIOMAC.2019.04.110>

576 Petraitytė, S., & Šipailienė, A. (2019). Enhancing encapsulation efficiency of alginate  
577 capsules containing lactic acid bacteria by using different divalent cross-linkers sources.  
578 *LWT*, 110, 307–315. <https://doi.org/10.1016/j.lwt.2019.01.065>

579 Pires, E. O., Caleja, C., Garcia, C. C., Ferreira, I. C. F. R., & Barros, L. (2021). Current status  
580 of genus *Impatiens*: Bioactive compounds and natural pigments with health benefits. *Trends*  
581 in Food Science & Technology. <https://doi.org/10.1016/J.TIFS.2021.01.074>

582 Pupa, P., Apiwatsiri, P., Sirichokchatchawan, W., Pirarat, N., Muangsin, N., Shah, A. A., &  
583 Prapasarakul, N. (2021). The efficacy of three double-microencapsulation methods for  
584 preservation of probiotic bacteria. *Scientific Reports* 2021 11:1, 11(1), 1–9.  
585 <https://doi.org/10.1038/S41598-021-93263-Z>

586 Raddatz, G. C., & Menezes, C. R. de. (2021). Microencapsulation and co-encapsulation of  
587 bioactive compounds for application in food: challenges and perspectives. *Ciência Rural*,  
588 51(3), 1–8. <https://doi.org/10.1590/0103-8478CR20200616>

589 Rezaei, M., ghasemi, Y., Sharifan, A., & Bakhoda, H. (2022). Producing and analyzing  
590 gamma-aminobutyric acid containing probiotic black grape juice using *Lactobacillus*  
591 *plantarum plantarum* IBRC(10817) and *Lactobacillus brevis* IBRC(10818). *Measurement:*  
592 *Food*, 8, 100056. <https://doi.org/10.1016/J.MEAFOO.2022.100056>

593 Rodriguez-Amaya, D. B. (2019). Betalains. *Encyclopedia of Food Chemistry*, 35–39.  
594 <https://doi.org/10.1016/B978-0-08-100596-5.21607-7>

595 Sabarez, H. (2021). Plant, Equipment and Utilities: Driers and Powder Packaging. Reference  
596 Module in Food Science. <https://doi.org/10.1016/B978-0-12-818766-1.00247-6>

597 Sadowska-Bartosz, I., & Bartosz, G. (2021). Biological Properties and Applications of  
598 Betalains. *Molecules*, 26(9). <https://doi.org/10.3390/MOLECULES26092520>

599 Sawicki, T., Bączek, N., & Wiczowski, W. (2016). Betalain profile, content and antioxidant  
600 capacity of red beetroot dependent on the genotype and root part. *Journal of Functional*  
601 *Foods*, 27, 249–261. <https://doi.org/10.1016/j.jff.2016.09.004>

602 Sharifi, S., Rezazad-Bari, M., Alizadeh, M., Almasi, H., & Amiri, S. (2021). Use of whey  
603 protein isolate and gum Arabic for the co-encapsulation of probiotic *Lactobacillus plantarum*  
604 and phytosterols by complex coacervation: Enhanced viability of probiotic in Iranian white  
605 cheese. *Food Hydrocolloids*, 113, 106496. <https://doi.org/10.1016/J.FOODHYD.2020.106496>

606 Sheu, T. Y., Marshall, R. T., & Heymann, H. (1993). Improving Survival of Culture Bacteria  
607 in Frozen Desserts by Microentrapment. *Journal of Dairy Science*, 76(7), 1902–1907.  
608 [https://doi.org/10.3168/jds.S0022-0302\(93\)77523-2](https://doi.org/10.3168/jds.S0022-0302(93)77523-2)

609 Silva, J. P. P., Bolanho, B. C., Stevanato, N., Massa, T. B., & Silva, C. da. (2020).  
610 Ultrasound-assisted extraction of red beet pigments (*Beta vulgaris* L.): Influence of  
611 operational parameters and kinetic modeling. *Journal of Food Processing and Preservation*,  
612 44(10), e14762. <https://doi.org/10.1111/JFPP.14762>

613 Shaaruddin, S., Ghazali, H. M., Hamed Mirhosseini, S., & Muhammad, K. (2017). Stability of  
614 betanin in pitaya powder and confection as affected by resistant maltodextrin. *LWT*, 84, 129–  
615 134. <https://doi.org/10.1016/J.LWT.2017.05.031>

616

617 Skalicky, M., Kubes, J., Shokoofeh, H., Tahjib-Ul-Arif, Md., Vachova, P., & Hejnak, V.  
618 (2020). Betacyanins and Betaxanthins in Cultivated Varieties of *Beta vulgaris* L. Compared to  
619 Weed Beets. *Molecules* 2020, Vol. 25, Page 5395, 25(22), 5395.  
620 <https://doi.org/10.3390/MOLECULES25225395>

621 Snigdha, S., Ha, K., Tsai, P., Dinan, T. G., Bartos, J. D., & Shahid, M. (2021). Probiotics:  
622 Potential novel therapeutics for microbiota-gut-brain axis dysfunction across gender and  
623 lifespan. *Pharmacology & Therapeutics*, 107978.  
624 <https://doi.org/10.1016/J.PHARMTHERA.2021.107978>

625 Spiegel, M., Gamian, A., & Sroka, Z. (2021). Antiradical Activity of Beetroot (*Beta vulgaris*  
626 L.) Betalains. *Molecules*, 26(9). <https://doi.org/10.3390/MOLECULES26092439>

627 Sravan Kumar, S., Singh Chauhan, A., & Giridhar, P. (2020). Nanoliposomal encapsulation  
628 mediated enhancement of betalain stability: Characterisation, storage stability and antioxidant  
629 activity of *Basella rubra* L. fruits for its applications in vegan gummy candies. *Food*  
630 *Chemistry*, 333, 127442. <https://doi.org/10.1016/J.FOODCHEM.2020.127442>

631 Teo, A., Lam, Y., Lee, S. J., & Goh, K. K. T. (2021). Spray drying of whey protein stabilized  
632 nanoemulsions containing different wall materials – maltodextrin or trehalose. *LWT*, 136,  
633 110344. <https://doi.org/10.1016/J.LWT.2020.110344>

634 Terpou, A., Papadaki, A., Lappa, I. K., Kachrimanidou, V., Bosnea, L. A., & Kopsahelis, N.  
635 (2019). Probiotics in Food Systems: Significance and Emerging Strategies Towards Improved  
636 Viability and Delivery of Enhanced Beneficial Value. *Nutrients*, 11(7).  
637 <https://doi.org/10.3390/NU11071591>

638 Tossi, V. E., Martínez Tosar, L., Pitta-Álvarez, S. I., & Causin, H. F. (2021). Casting light on  
639 the pathway to betalain biosynthesis: A review. In *Environmental and Experimental Botany*  
640 (Vol. 186, p. 104464). Elsevier B.V. <https://doi.org/10.1016/j.envexpbot.2021.104464>

641 Vivek, K., Mishra, S., & Pradhan, R. C. (2020). Characterization of spray dried probiotic  
642 Sohiong fruit powder with *Lactobacillus plantarum*. *LWT*, 117.  
643 <https://doi.org/10.1016/J.LWT.2019.108699>

644 Westfall, S., Carracci, F., Estill, M., Zhao, D., Wu, Q., Shen, L., Simon, J., & Pasinetti, G. M.  
645 (2021). Optimization of probiotic therapeutics using machine learning in an artificial human  
646 gastrointestinal tract. *Scientific Reports*, 11(1). <https://doi.org/10.1038/S41598-020-79947-Y>

647 Yang, W., Kaimainen, M., Järvenpää, E., Sandell, M., Huopalahti, R., Yang, B., &  
648 Laaksonen, O. (2021). Red beet (*Beta vulgaris*) betalains and grape (*Vitis vinifera*)  
649 anthocyanins as colorants in white currant juice – Effect of storage on degradation kinetics,  
650 color stability and sensory properties. *Food Chemistry*, 348.  
651 <https://doi.org/10.1016/J.FOODCHEM.2020.128995>

652 Yoha, K. S., Nida, S., Dutta, S., Moses, J. A., & Anandharamakrishnan, C. (2021). Targeted  
653 Delivery of Probiotics: Perspectives on Research and Commercialization. *Probiotics and*  
654 *Antimicrobial Proteins*, 1. <https://doi.org/10.1007/S12602-021-09791-7>

655 Zavaleta, E. B., Coavichi, L. I. L., Rodríguez, L. C. V., Andrade, E. F., García, H. S., &  
656 Rascón Díaz, M. P. (2022). Co-microencapsulation of *Lactobacillus rhamnosus* and krill oil  
657 by spray-drying. *Food Bioscience*, 50, 102133. <https://doi.org/10.1016/J.FBIO.2022.102133>

658 Zendeboodi, F., Khorshidian, N., Mortazavian, A. M., & da Cruz, A. G. (2020). Probiotic:  
659 conceptualization from a new approach. *Current Opinion in Food Science*, 32, 103–123.  
660 <https://doi.org/10.1016/J.COFS.2020.03.009>

661 Zhang, W., Sun, Z., Bilige, M., & Zhang, H. (2015). Complete genome sequence of probiotic  
662 *Lactobacillus plantarum* P-8 with antibacterial activity. *Journal of Biotechnology*, 193, 41–42.  
663 <https://doi.org/10.1016/j.jbiotec.2014.11.011>

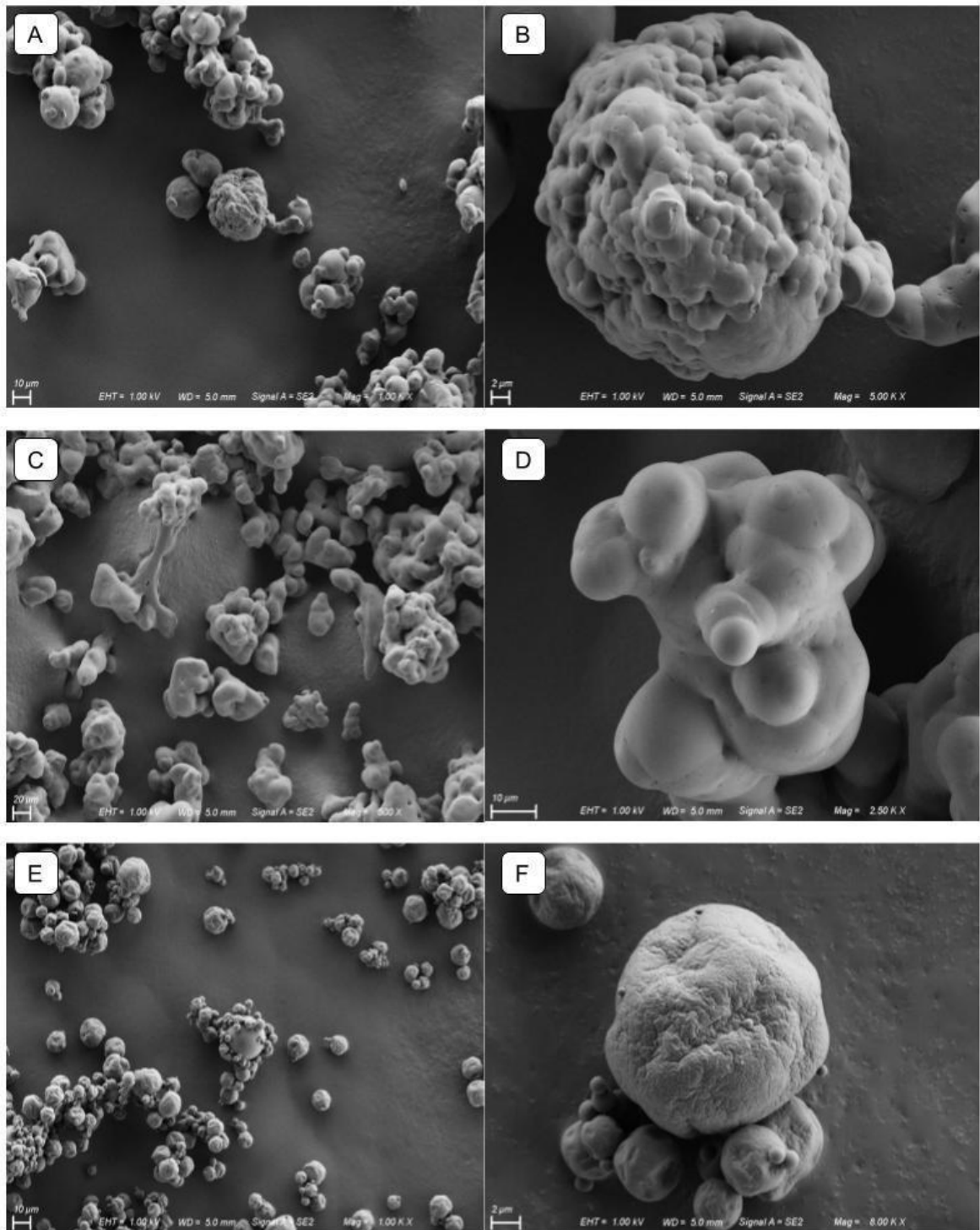
664

665

666

667

668



669

670

Figure 1 - Optical microscopy of probiotic microcapsules with and without betalains extract:

671

T3 (A=1000x and B=5000x): standard microcapsule containing *L. plantarum* without betalainas

672

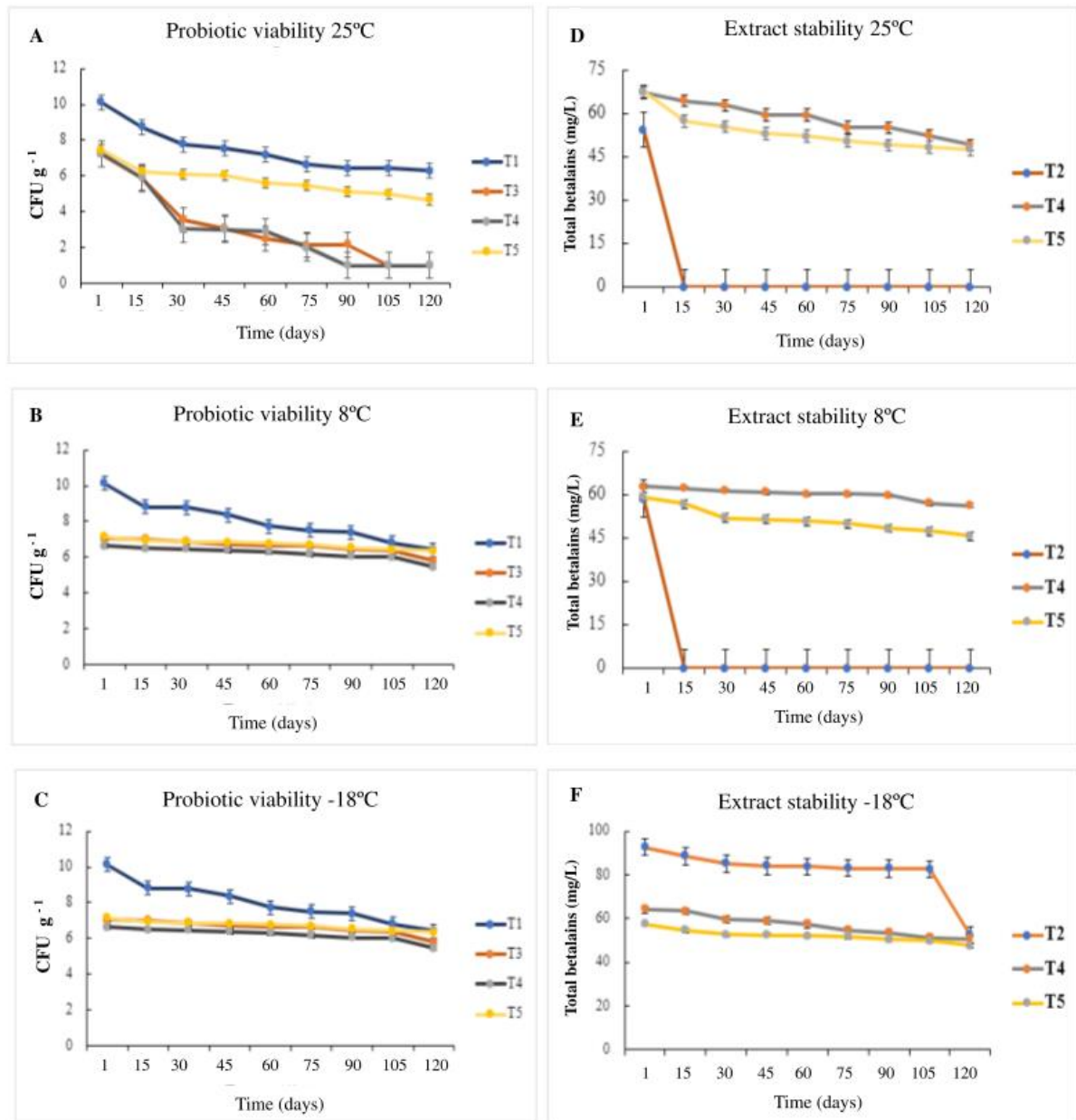
extract; T4 (C=500x and D=2500x): microcapsule containing *L. plantarum* with 100%

673

betalainas extract; T5 (E=1000x and F=8000x): microcapsule containing *L. plantarum* with

674

50% betalainas extract.



675

676

Figure 2 - Viability and stability of free and microencapsulated *L. plantarum* and betalains upon

677

storage. T1= cell culture in the free form of *L. plantarum*, T2= free extract, T3= microcapsules

678

with *L. plantarum* without extract, T4= microcapsules with *L. plantarum* with 100% beet stem

679

extract and T5= microcapsules with *L. plantarum* with 50% beet stem extract.

680

681

682

683

684

685

686

687

688

689

690

691

692

693

694

695

696

697

698

699

700

701

702

703

704

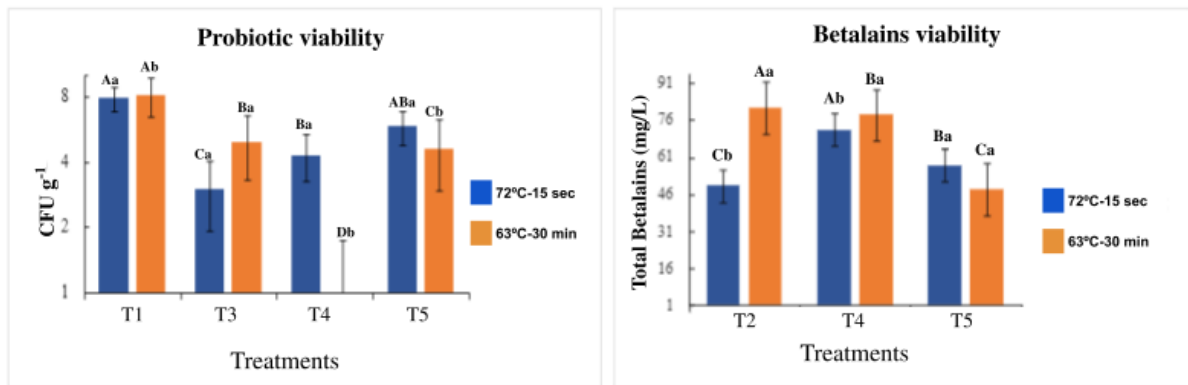


Figure 3. Viability of microencapsulated and free *L. plantarum* and betalainas to heat treatment, where, the difference statistically (Tukey test,  $p < 0.05$ ) between treatments is represented by different capital letters and different lower case letters represent the difference between simulated temperatures. T1= cell culture in the free form of *L. plantarum*, T2= free extract, T3= microcapsules with *L. plantarum* without extract, T4= microcapsules with *L. plantarum* with 100% beet stem extract and T5= microcapsules with *L. plantarum* with 50% beet stem extract.

705

706

707

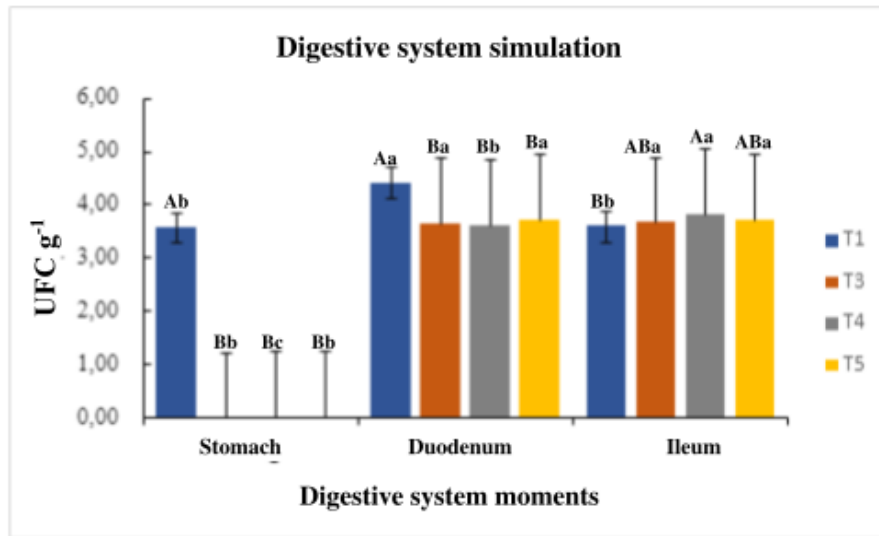
708

709

710

711

712



713

714

715

716

717

718

719

720

721

722

723

724

725

726

727

728

Image 4. Viability of microencapsulated and free *L. plantarum* in the digestive system simulation, where, the statistically difference (Tukey's test,  $p < 0.05$ ) between treatments is represented by different upper and lower case letters represents the difference between the stages of gastric simulation. T1= cell culture in the free form of *L. plantarum*, T3= microcapsules with *L. plantarum* without extract, T4= microcapsules with *L. plantarum* with 100% beet stem extract and T5= microcapsules with *L. plantarum* with 50% beet stem extract.

729 Table 1 - Composition of treatments with and without microencapsulation.

Reagents	Treatments				
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>
Arabic Gum	-	-	4 g	4 g	4 g
Trehalose	-	-	4 g	4 g	4 g
Maltodextrin	-	-	2 g	2 g	2 g
Twen 80	-	-	0.1 mL	0.1 mL	0.1 m
Glycerol	-	-	1.90 mL	1.90 mL	1.90 mL
Probiotic culture	1 g	-	1 g	1 g	1 g
Betalains extract	-	100 mL	-	100 mL	50 mL
Water	-	-	100 mL	-	50 mL
Final solids concentration (m / v):				~ 12%	

730

731 T1= cell culture in free form of *L. plantarum*, T2= beet stem extract in free form, T3= microcapsules

732 with *L. plantarum* without extract, T4= microcapsules with *L. plantarum* with 100% beet stem extract

733 and T5= microcapsules with *L. plantarum* with 50% beet stem extract.

734

735

736

737

738

739

740

741

742

743

744 Table 2 - Viability, average microparticle size and encapsulation efficiency.

Treatments	Post-encapsulation viability				
	Probiotic CFU g <sup>-1</sup>	Extract mg / L	Average particle size (µm)	EE % Probiotic	EE % Extract
T1 (initial)	*10.11 ± 0,03 <sup>a</sup>	-	-	-	-
T2	-	92.61 ± 0,00 <sup>a</sup>	-	-	-
T3	7.22 ± 0,05 <sup>b</sup>	-	5.76 ± 1,08 <sup>c</sup>	71.43 ± 0,65 <sup>a</sup>	-
T4	7.24 ± 0,18 <sup>b</sup>	64.27 ± 0,02 <sup>b</sup>	27.30 ± 0,00 <sup>a</sup>	71.56 ± 1,68 <sup>a</sup>	69.39 ± 0,02 <sup>a</sup>
T5	7.44 ± 0,05 <sup>b</sup>	57.52 ± 0,07 <sup>c</sup>	10.20 ± 0,00 <sup>b</sup>	73.57 ± 0,67 <sup>a</sup>	62.11 ± 0,08 <sup>b</sup>

745 Averages followed by different lower case letters differ statistically in the column (Tukey's test,  
 746 p <0.05). T1= cell culture in free form of *L. plantarum*, T2= beet stem extract in free form, T3=  
 747 microcapsules with *L. plantarum* without extract, T4= microcapsules with *L. plantarum* with  
 748 100% beet stem extract and T5= microcapsules with *L. plantarum* with 50% beet stem extract.

749  
 750  
 751