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Calabuig-Jiménez, L.; Betoret, E.; Betoret Valls, N.; Patrignani, F.; Barrera Puigdollers, C.; Seguí Gil, L.; Lanciotti, R.... (2019). High pressures homogenization (HPH) to microencapsulate L. salivarius spp. salivarius in mandarin juice. Probiotic survival and in vitro digestion. Journal of Food Engineering. 240:43-48. https://doi.org/10.1016/j.jfoodeng.2018.07.012

The final publication is available at

https://doi.org/10.1016/j.jfoodeng.2018.07.012

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Additional Information

High pressures homogenization (HPH) to microencapsulate *L. salivarius* spp. *salivarius* in mandarin juice. Probiotic survival and *in vitro* digestion

Calabuig-Jiménez, Laura[1]; Betoret, Ester[2*]; Betoret, Noelia[1]; Patrignani, Francesca[2,3]; Barrera, Cristina[1]; Seguí, Lucía[1]; Lanciotti, Rosalba[2,3]; Dalla Rosa, Marco[2,3]

[1] Instituto de Ingeniería de Alimentos para el desarrollo, Universitat Politècnica de València, Valencia, Spain.

[2] Department of Agricultural and Food Sciences, University of Bologna, Cesena, Italy.

[3]Interdepartmental Centre for Agri-Food Industrial Research, University of Bologna, Cesena, Italy.

*Corresponding author: Ester Betoret (ester.betoret@iata.csic.es)

1. Introduction

The importance of the microbiome in the incidence of a large number of diseases becomes evident; from infectious diseases to degenerative diseases, including cancer, obesity and even psychological diseases (Avershina et al., 2017; Auderson et al., 2017; Subramanyan et al, 2017; Rouxinol-Dias, 2016). Together with this, it has been demonstrated that food can influence growth, viability and survival of microorganisms in gastrointestinal tract thus conditioning the human organism microbiota and therefore recommending probiotic food consumption (Kashtanova et al., 2016).

Dairy products are more suited to probiotic food development. However, due to the high prevalence of lactose intolerance, different non-dairy probiotic products such as fruit juices, cereal based breakfast products and baby foods have been developed in recent years (Anekella & Orsat, 2013; Chen & Mustapha, 2012; Rivera-Espinoza & Gallardo-Navarro, 2010). In any case, there is a need for designing new products which can deliver between 10^7 - 10^9 viable cells into the intestine by consuming approximately 100 g/day of the product (Rad et al., 2013).

Mandarin juice is quite appreciated by its functional properties due to the presence of antioxidants and phenolic compounds such as hesperidin, carotenoids and vitamin C (Putnik et al., 2017). Those

bioactive compounds of mandarin juice have been related with a health promoting effect against cancer, hypertension, cardiovascular disorders, stroke and diabetes (Milella et al., 2011; Jedrychowski et al., 2010). Beside this, fermented citrus juices can have antibacterial activities (Hashemi et al., 2017). Concretely, *Lactobacillus salivarius* spp. *salivarius* has a demonstrated probiotic effect (Aiba et al., 1998) with antagonist properties against *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteriditis* (Betoret et al., 2017).

It has been demonstrated in numerous research works that not only food matrix, processing conditions and storage time, but also digestion process clearly influence the total amount of probiotic microorganisms able to reach the targeted tissue (Sagdic et al., 2012). Therefore, product formulation and process conditions should be directed to increase probiotic resistance to stress conditions and to improve viability, acid and bile tolerance, adhesion to intestinal epithelium, antimicrobial properties, antibiotic resistance and other functionality of probiotics that determine their efficacy in the gastrointestinal tract.

Microencapsulation is one of the most efficient strategies that has been considered in recent years to protect probiotic cells from degradation by adverse conditions, and to control their release under particular conditions (Martín et al., 2015). In fact, during the past few years, a number of food products containing encapsulated probiotics cells have been introduced into the market (Burgain et al., 2011). Although the most used microencapsulation techniques are extrusion, freeze and spray drying there is a need to develop more competitive technologies with industrial applications (Vinceković, 2017 et al., 2017; Mota et al., 2018). Burns et al. (2008) used high pressure homogenization (HPH) ranging between 60 and 100 MPa to increase *Lb. paracasei* A13 and *Lb. acidophilus* 08 viability in probiotic fermented milks and cheeses. Tabanelli et al. (2013) and Betoret et al. (2017) demonstrated that sub-lethal HPH treatment (performed at 50 MPa) improved functional properties of probiotic bacteria (such as hydrophobicity, auto-aggregation and resistance to biological stresses) in different food matrixes and preserved their viability during refrigerated storage. Patrigniani et al. (2017) used HPH at 50 MPa to microencapsulate *L. paracasei* A13 and *L. salivarius* spp. *salivarius* CECT 4063 to produce functional fermented milks. This technology

already implemented at industrial level to improve quality attributes of fruit juices could be used to microencapsulate probiotics and increase viability in citrus juices.

The aim of this research was to determine the effect of *Lactobacillus salivarius* spp. *salivarius* microencapsulation, by using high pressure homogenization, on the probiotic survival under simulated gastrointestinal conditions when incorporated into mandarin juice and stored. Physicochemical and technological properties of mandarin juice were also evaluated.

2. Material and methods

2.1. Strain and food materials

Lactobacillus salivarius spp. *salivarius* CECT 4063 was obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain).

Mandarin fruit cv. Ortanique (Citrus sinensis x Citrus reticulata) was provided by Rural S. Vicent Ferrer cooperative located in Benaguacil, Valencia, Spain. Juice preparation was done following the procedure described in WO/2007/042593. Fruits were washed, drained, squeezed ("GAM" MOD.SPA 1400 rpm, power 350W – monophase 220V, Cesena, Italy) filtered with 0.7 mm sieve, centrifuged at 3645 x g during 5 minutes at 5ºC (Beckman Coulter AvantiTM J-25, California, United States) and pasteurized at 63 ºC for 15 s (Roboqbo Qb8-3, Bologna, Italy) (Izquierdo et al., 2007).

2.2. Microencapsulation procedure

To microencapsulate L. salivarius spp. salivarius the method described by (Ding & Shah, 2009) was followed with some modifications. A volume of 2 L of Man, Rogosa & Sharpe (MRS) Broth (Scharlab, Barcelona, Spain) containing 10^9 CFU/mL of *Lactobacillus salivarius* spp. *salivarius* was centrifuged at 7700 x g for 15 mins at 10ºC (Beckman Coulter AvantiTM J-25, California, United States) and suspended in 100 mL sterile water. A mixture of 25 mL of microorganism solution, 100 mL of sodium alginate (3%) (Sigma-aldrich, Steinheim, Germany), 1 mL of tween 80 (Sharlau, Sentmenat, Spain) and 200 mL of commercial sunflower oil was homogenized in two passes through the valve at 70 MPa and at room temperature with a homogenizer (Panda Plus Niro Soavi, Parma, Italy). The emulsion was broken with calcium chloride 0.1 M (Sigma-aldrich, Steinheim, Germany)

and kept overnight at 4 ºC to separate the phases. Microcapsules were isolated by centrifugation at 8000 rpm (7700 x g) for 15 minutes at 10ºC (Beckman Coulter AvantiTM J-25, California, United States).

2.3. Mandarin juice with probiotic microorganisms

Mandarin juice with non-encapsulated *L. salivarius* spp. *salivarius* was prepared following the methodology described in Betoret et al. (2012) by inoculation with 4 mL/L of MRS broth (Scharlab, Barcelona, Spain) containing 10^9 CFU/mL and maintained at 37 ºC for 24 h. Prior to this step, the juice pH was modified by adding 9.8 g/L of sodium bicarbonate (Hacendado, Novelda, Spain).

Mandarin juice with microencapsulated *L. salivarius* spp. *salivarius* was prepared by adding microcapsules prepared as described above into the juice at a ratio of 1.45 juice/microcapsules (w/w). The mixture was maintained in agitation at room temperature for 1 h.

2.4. Physicochemical characterization

Total soluble solids (ºBrix) was measured with a digital refractometer (DR 201-95 A.KRUSS OPTRONIC, Hamburg, Germany) at 20 ºC, and pH with a pH meter (Crison GLP21, Barcelona, Spain). A liquid pycnometer was used to determine the density. Water activity was measured using a dew point hygrometer (DECAGÓN Aqualab CX-2, Washington, United States). The values provided are the average of three replicates.

2.5. Particle size

Particle size was determined with a Mastersizer 2000 equipment (Malvern Instruments, Worcestershire, UK) following the methodology described by Betoret et al., (2009) with some modifications. The refractive indexes used were 1.73, 1.33 and 1.46 (Ciron et al., 2010), the absorption index of cloud particles were 0.1 (Correding et al., 2001) and 0.01 (Ciron et al., 2010) for non-encapsulated and encapsulated L. salivarius spp. salivarius mandarin juices respectively. Results were expressed as the volume-weighted mean diameter (D [4,3]), the surface area mean diameter (D [3,2]) and d_{10}, d_{50} and d_{90}, defined as the particle size which 10%, 50% and 90% of the distribution

is below this size respectively (Instruments, M., 2007). The values provided are the average of five replicates.

2.6. Rheological properties

Rheological properties were studied with a rheometer (Haake RheoStress 1, Thermo Electron Corporation, Kalsruhe, Germany) using a concentric cylinder (Z34 DIN Ti, Thermo Electron Corporation, Kalsruhe, Germany). Controlled shear rate experiments were done for 300 s with an increasing rate from 0 to 250s^{-1} at 20 ºC. Parameters K (consistency index, Pa·s) and n (flow behaviour index, dimensionless) were obtained by regression adjusted to Ostwald-de-Waele model linearized as equation 1, where σ (Pa) is the shear stress, K is the consistency index, γ (s^{-1}) is the shear rate and n is the flow behavior index. HAAKE RheoWin Data Manager v.3.61.0004 software was used to process data. The values provided are the average of three replicates.

$$\sigma = K \cdot \gamma^{n} \quad (1)$$

2.7. Microbial content

Mandarin juices with encapsulated and non-encapsulated *L. salivarius* spp. *salivarius* were stored at 4 ºC and microbial survival was evaluated at 0, 1, 3, 7 and 10 days. Microbial content was determined following the dilution method and growth in MRS agar (Scharlab, Barcelona, Spain) on double layer incubated during 24 h at 37 ºC. In juice with encapsulated *L. salivarius* spp. *salivarius* the first dilution was done in phosphate buffer solution (pH 7.4) maintained in agitation during 30 minutes. Values provided are the average of four replicates.

2.8. Gastrointestinal digestion

In order to determine the effect of gastrointestinal digestion on the microorganism survival two variables were considered: t_i referred to a moment during the gastrointestinal digestion; T_i referred to the *L. salivarius* spp. *salivarius* content at different stages during the gastrointestinal digestion. A dilution 1:1 of the mandarin juice with 0.6% (w/v) pepsine (Sigma-aldrich, Steinheim, Germany) was adjusted with HCl 4M to pH 3 (t_1 - T_1). Sample was kept in an agitated bath at 37 ºC for 90

minutes (t_2 - T_2). Phosphate buffer solution at pH 8 with 10% of bile (Sigma-aldrich, Steinheim, Germany) were added and mixed (t_3 - T_3). Finally, phosphate buffer solution at pH 8 with 0.3% of bile 0.1% pancreatine (Sigma-aldrich, Steinheim, Germany) was added and sample was incubated at 37 ºC for 90 minutes (t_4 - T_4). Microorganism content was measured by plate count after each of the four stages considered for gastrointestinal digestion process described before. The results provided are the average of four replicates.

2.9. Statistical analysis

A multi factorial ANOVA was carried out to determine the significant effect of the process variables, at 95% confidence level, using Statgraphics centurion XVI software (StatPoint Technologies, Virginia, US).

3. Results and discussion

3.1. Physicochemical characterization, particle size and rheological properties

Minor proportion of mandarin juice together with the microcapsules incorporated were responsible for the minor total soluble solids content obtained in mandarin juice with encapsulated *L. salivarius* spp. *salivarius* (table 1).

Particle size distribution of all samples ranged between 0.5 and 1500 µm (figure 1). The wideness of the distribution and the variability in the particle sizes obtained could be due to the presence of different cloud particles such as cellular organelles and membranes, oil droplets, chromoplasts, fragments of cellular wall such as pectin, cellulose and hemicellulose and functional compounds (Baker & Cameron, 1999). Fresh mandarin juice and mandarin juice with non-encapsulated *L. salivarius* spp. *salivarius* showed a bimodal distribution. Fresh mandarin juice showed a maximum peak at 7.6 µm and a minimum peak at 416.6 µm. Mandarin juice with non-encapsulated *L. salivarius* spp. *salivarius* showed a maximum peak at 19.9 µm and a minimum peak at 724.4 µm. Despite of *L. salivarius* spp. *salivarius* microbial cells sizes varies between 1 and 8 µm (Kokkinosa et al., 1998), their presence increased slightly the particle size distribution of mandarin juice. This result could evidence an interaction and aggregation of juice cloud particles promoted by the presence of

169 microorganisms. Particle size distribution of the microcapsules was monomodal, with a maximum
170 peak at 316.3 µm. The addition of the microcapsules to the mandarin juice changed the distribution
171 from bimodal to monomodal with a maximum peak at 316.3 µm too. A possible aggregation of the
172 microcapsules with the suspended particles of the mandarin juice could explain these results.

173 Table 2 shows values of the main parameters that describe particle size distribution. Differences
174 obtained between D(4,3) and D(3,2) values in both mandarin juices evidenced the existence of
175 particles with high variability in shape and size. Particle size is an important parameter to be
176 considered when mandarin juice enriched with microcapsules is going to be consumed directly and
177 or when it is going to be used in other pretreatment operations such as vacuum impregnation.
178 Microcapsules smaller than 100 µm are required in order to do not be perceived by the consumer
179 (Hansen, et al., 2002). In vacuum impregnation operation, a particle size smaller than the food matrix
180 porous is required (Castagnini et al., 2015). Patrignani et al., (2017) showed that high pressure
181 homogenization at 50 MPa allows obtaining microcapsules of *Lactobacillus* microorganisms such as
182 *L. paracasei* and *L. salivarius* smaller than 100 µm. In our case, less than 50% of the particles in the
183 mandarin juice with encapsulated *L. salivarius* spp. *salivarius*, had a size smaller than 100 µm (figure
184 1). Nevertheless, results of d_{50} in mandarin juice with encapsulated *L. salivarius* spp. *salivarius*
185 revealed that the microcapsules obtained by homogenization pressures were similar to those obtained
186 by other traditional microencapsulation methods such as spray drying, spray cooling, spray chilling,
187 extrusion, freeze-drying and coacervation (Desai and Park, 2005; Ding & Shah, 2009, Gibbs et al.,
188 1999; Gouin, 2004; Shahidi and Han, 1993).

189 Microcapsules incorporation had an impact on mandarin juice rheological behavior. In fact, the
190 rheological obtained curves showed that encapsulated *L. salivarius* spp. *salivarius* mandarin juice
191 resulted in a more viscous fluid than non-encapsulated one. Experimental data were fitted to the
192 Ostwald-de-Waele model (table 3). A Newtonian behavior is generally observed for clarified and
193 depectinated orange juices (Ibarz et al., 1994). In our case, both fluids resulted in a non-Newtonian
194 pseudo plastic behavior ($n<1$) generally observed in complex fluids or polymer solutions in which
195 viscosity decreases under shear strain. Rheological properties of the isolated microcapsules were not
196 characteristic of a liquid because of the irregular aggregates formed.

197 *3.2. Probiotic survival during storage and gastrointestinal digestion effect*

198 In order to have a probiotic effect or any other beneficial effect associated to the microorganism
199 strain it is necessary, firstly, to maximize the microorganism content and its survival in the food
200 matrix during all the processing and storage conditions; then, the microorganism needs to maintain
201 its active form after the consumption and during all digestion steps until the targeted site where it
202 will be able to interact, colonize and finally will exert its beneficial effect. As described in Betoret et
203 al., (2016), *Lactobacillus* cells survival in mandarin juice is affected mainly by low pH, high
204 temperature, hyperosmotic stress, nutrient bioavailability, cloud structure and stability.

205 Content of *L. salivarius* spp. *salivarius* encapsulated and non-encapsulated was determined in
206 mandarin juice after 1, 3, 7 and 10 storage days. Results are shown in table 4 (T_0). Despite of
207 differences obtained in both microorganism content at day 1, no significant differences were
208 observed at 3 and 7 storage days. After 10 storage days, the content of encapsulated *L. salivarius*
209 spp. *salivarius* was significantly higher than non-encapsulated one. It seems that entrapment of *L.*
210 *salivarius* spp. *salivarius* by a microcapsule formed by homogenization pressures and with alginate
211 as a coating it is protective enough to increase significantly ($p \leq 0.05$) its survival in mandarin juice
212 at 10 storage days.

213 *L. salivarius* spp. *salivarius* has been proved to have both, effect against *Helicobacter pylori*
214 infection and probiotic (Messaoudi, et al., 2013, Zheng, et al., 2013). *L. salivarius* spp. *Salivarius*
215 probiotic effect could be improved when added to mandarin juice, because of a synergic effect
216 between the flavanones of the juice and the probiotic bacteria (Pereira-Caro et al., 2015; Putignani
217 & Dallapiccola, 2016). The precise mechanisms by which probiotic microorganisms have an effect
218 against *Helicobacter pylori* infection are still unknown. A possible competition over the binding sites
219 in the gastrointestinal tract between the probiotic and the bacteria and a posterior displacement by
220 the probiotic is widely accepted. There are evidences that *L. salivarius* spp. *salivarius* colonizes the
221 stomach and produce immunomodulatory factors which suppress inflammation caused by *H. pylori*
222 infection of the gastric epithelial cells (Aiba et al., 1998, Servin 2004, Panpetch et al., 2016). In this
223 case, it will be necessary that *L. salivarius* spp. *salivarius* maintain its active form until the stomach
224 where it will be able to compete with Helicobacter pylori bacteria and interact with gastric epithelial

tissue in order to exert a positive effect against infection. Nevertheless, in order to have a probiotic effect will be necessary that *L. salivarius* spp. *salivarius* maintains its active form until reaching the intestine where must be able to interact with intestine wall to carry out a subsequent colonization. In both cases, microcapsule function is twofold, on the one hand protecting *L. salivarius* spp. *salivarius* enough to resist unfavorable conditions during digestion process but on the other hand allowing the release at the appropriate time and point in the organism so that it can interact with the target tissue. Simulated gastrointestinal digestion was carried out in order to know the survival of *L. salivarius* spp. *salivarius* encapsulated and non-encapsulated in mandarin juice.

The microbial content during gastrointestinal simulation is shown in table 4. T_0 means the initial content of *L. salivarius* spp. *salivarius* in mandarin juice. T_1 and T_2 refer to the microorganism quantity by simulated stomach conditions after pH change by HCl addition and peristaltic movements respectively. T_3 and T_4 are the counting of microorganism after the duodenal shock and intestinal juice mixing respectively. Statistical analysis revealed that all variables studied; the encapsulation, the specific moment in the simulated gastrointestinal digestion and the storage time had a significant effect ($p \leq 0.05$) on *L. salivarius* spp. *salivarius* content. Figure 3 shows the evolution of the microbial concentration (T_i/T_0) throughout the gastrointestinal digestion process (t_i) in the stored juices. Thus, probiotic resistance to the digestion process was influenced by juice storage time. During three storage days, the encapsulation of the probiotic increased its resistance from t_2. However, when the juice was stored for 7 and 10 days, the positive effect of the capsule on the microorganism survival was evident from t_1. In order to quantify the effect of the different factors, the percentage of accumulated degradation was calculated (table 5). Microorganism encapsulation caused a decrease in the degradation percentage from 8-9% to 0-2% when the juice was stored for 7 to 10 days. After mixing simulating peristaltic stomach movements, the accumulated degradation was independent of microencapsulation and storage time. The biggest differences were observed in the passage from the stomach to the intestine. Thus, the duodenal shock resulted in degradation percentages between 18 and 30% in the mandarin juice with encapsulated *L. salivarius* spp. *salivarius*. Degradation percentages increased to 42 and 72% in mandarin juice with non-encapsulated microorganisms. Simulated gastrointestinal digestion resulted in losses around 50% in

253 encapsulated *L. salivarius* spp. *salivarius* increasing to levels of 75-85% in non-encapsulated
254 microorganisms. Similar results were obtained by Abbaszadeh et al., (2013). However, Gandoni et
255 al. (2016) obtained lower rate survival in apple juices enriched with *L. rhamnosus* encapsulated and
256 non.

257 The efficiency of the encapsulation method and the stability of the protective material could
258 explain the obtained results. Ding and Shah (2009), observed a microencapsulating efficiency of 77%
259 when capsules were generated by a microfluidizer at 68 MPa. A similar efficiency in our method
260 could explain the 20% of degradation, affecting non-encapsulated *L. salivarius* spp. *salivarius*,
261 produced in the acid stages of the simulated gastrointestinal digestion process. Beside this, the
262 solubility of alginate salts at pH above 3.5 could leave encapsulated microorganims unprotected in
263 the last stage of the gastrointestinal digestion. The values observed in t_2 and t_3 (figure 3) could be
264 explained considering that non-encapsulated microorganisms have been degraded by the acidic
265 conditions but microcapsules has not had enough time to be solubilized.

266

267 **4. Conclusion**

268 Microencapsulation by homogenization at pressures of 70 MPa with alginate as a coating seems
269 to be a promising strategy to protect *L. salivarius* spp. *salivarius* during gastrointestinal digestion
270 process and storage. The efficiency of the encapsulation method together with the stability of the
271 protective material could explain the obtained results in the simulated gastrointestinal digestion.

272 The incorporation of encapsulated *L. salivarius* spp. *salivarius* into mandarin juice modified its
273 physicochemical and technological properties creating a complex food matrix with new aggregates
274 and interactions that will need to be analyzed in further studies

275

276 **5. Acknowledgments**

277 This research was supported by a Marie Curie Intra European Fellowship (626643) within the 7th
278 European Community Framework Programme. Authors also acknowledge the FPI–UPV programme
279 and the FPI-mobility grant of the Universitat Politècnica de València.

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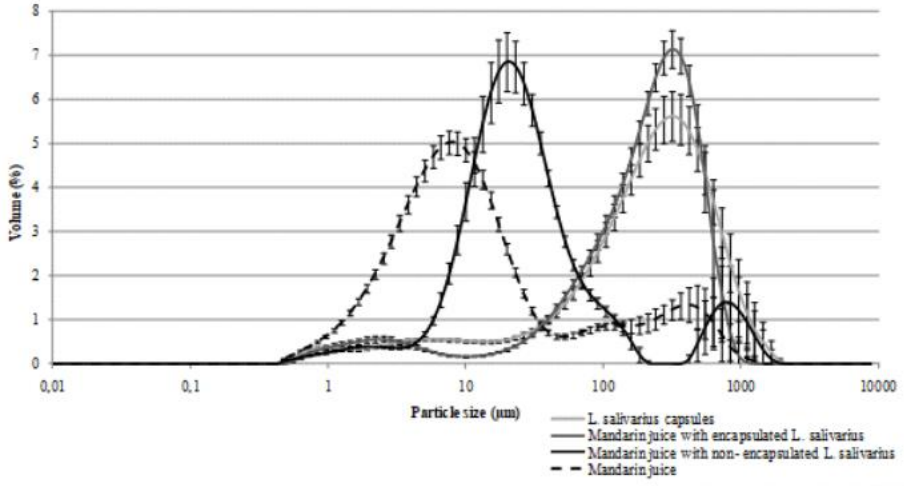

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419 **Figure 1**

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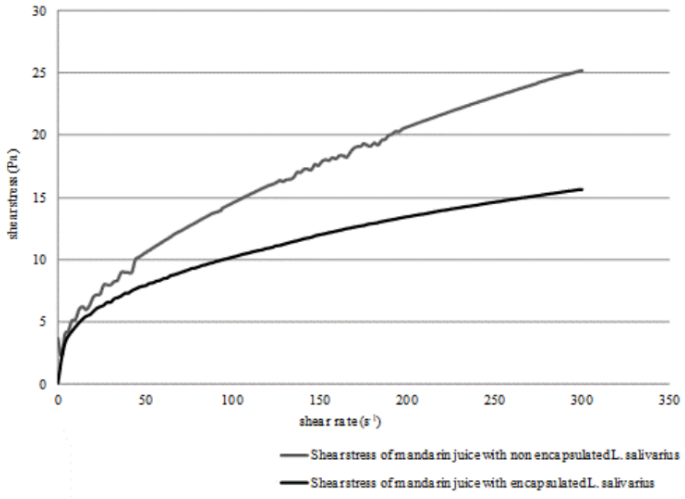
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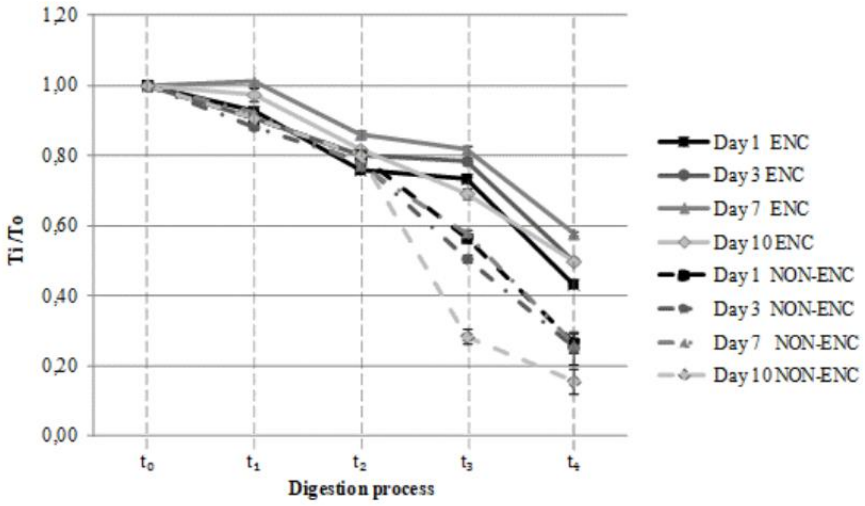
Shear stress of mandarin juice with non encapsulated L. salivarius
Shear stress of mandarin juice with encapsulated L. salivarius

Figure 2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Digestion process
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Day 10 NON-ENC

Figure 3

FIGURE CAPTIONS

Figure 1. Particle size distribution for the capsules, the mandarin juice with encapsulated *L. salivarius* spp. *salivarius*, the mandarin juice with non-encapsulated *L. salivarius* spp. *salivarius* and the mandarin juice.

Figure 2. Rheogram of mandarin juice with encapsulated *L. salivarius* spp. *salivarius* and mandarin juice with non-encapsulated *L. salivarius* spp. *salivarius*.

Figure 3. Evolution of encapsulated and non-encapsulated *L. salivarius* spp. *salivarius* in mandarin juice during the digestion process at 1, 3, 7 and 10 days.

Table 1. Physicochemical properties of the mandarin juice with encapsulated and non-encapsulated *L. salivarius* spp. *salivarius*. Values expressed as mean ± standard deviation.

	Non-encapsulated	Encapsulated
TSS (ºBrix)	13.63 ± 0.06^{a}	9.8 ± 0.2^{b}
pH	3.7 ± 0.01^{a}	3.4 ± 0.01^{b}
a_w	0.989 ± 0.003^{a}	0.994 ± 0.003^{a}
Density (g/mL)	1.060 ± 0.001^{a}	1.033 ± 0.008^{b}

Values with different superscript letters in a row are significantly different ($p \leq 0.05$)

Table 2. Characteristic parameters that describe particle size distribution of the mandarin juices and the capsules. Values expressed as mean ± standard deviation.

	D[4,3]	D[3,2]	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)
Mandarin juice	74 ± 30^{a}	5.9 ± 0.3^{a}	2.50 ± 0.09^{a}	10 ± 0.8^{a}	280 ± 64^{a}
Mandarin juice with non-encapsulated *L. salivarius* spp. *salivarius*	177 ± 83^{b}	13.9 ± 1.3^{b}	8.4 ± 0.8^{b}	31 ± 17^{b}	577 ± 295^{b}
Mandarin juice with encapsulated *L. salivarius* spp. *salivarius*	265 ± 28^{c}	22 ± 3^{c}	29 ± 10^{c}	235 ± 20^{c}	543 ± 63^{b}
Capsules of *L. salivarius* spp. *salivarius*	317 ± 46^{d}	20.9 ± 1.8^{c}	14.9 ± 3^{d}	240 ± 25^{c}	721 ± 129^{c}

Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

Table 3. Rheological properties of mandarin juice with encapsulated and non-encapsulated *L. salivarius* spp. *salivarius*. Values expressed as mean ± standard deviation.

	Non-encapsulated	Encapsulated
K (Pa·s)	1.96 ± 0.07^{a}	1.92 ± 0.04^{a}
n	0.376 ± 0.007^{a}	0.463 ± 0.004^{b}

Values with different superscript letters in a row are significantly different ($p \leq 0.05$)

Table 4. *L. salivarius* spp. *salivarius* content (log CFU/L) of mandarin juice with and without the encapsulated microorganisms during in vitro digestion over ten days. Values expressed as mean ± standard deviation.

		Day 1	Day 3	Day 7	Day 10
Encapsulated	T_0	9.09 ± 0.03^{j}	7.93 ± 0.05^{h}	6.87 ± 0.05^{h}	6.64 ± 0.06^{g}
	T_1	8.419 ± 0.016^{i}	7.18 ± 0.05^{f}	6.92 ± 0.08^{h}	6.47 ± 0.04^{g}
	T_2	6.89 ± 0.02^{f}	6.34 ± 0.08^{d}	5.91 ± 0.04^{f}	$5.43 \pm 0.02^{e,f}$
	T_3	6.66 ± 0.04^{e}	6.22 ± 0.04^{d}	5.61 ± 0.05^{e}	4.59 ± 0.06^{d}
	T_4	3.93 ± 0.04^{b}	3.96 ± 0.07^{b}	3.96 ± 0.02^{c}	3.31 ± 0.07^{c}
Non-encapsulated	T_0	8.08 ± 0.05^{h}	7.53 ± 0.07^{g}	6.14 ± 0.04^{g}	5.65 ± 0.05^{f}
	T_1	7.32 ± 0.03^{g}	6.637 ± 0.014^{e}	5.66 ± 0.05^{e}	5.12 ± 0.04^{e}
	T_2	6.48 ± 0.09^{d}	5.892 ± 0.017^{c}	4.74 ± 0.07^{d}	4.52 ± 0.02^{d}
	T_3	4.56 ± 0.05^{c}	3.81 ± 0.04^{b}	3.56 ± 0.04^{b}	1.60 ± 0.12^{b}
	T_4	2.1 ± 0.2^{a}	1.9 ± 0.3^{a}	1.60 ± 0.12^{a}	0.9 ± 1.0^{a}

Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

585 **Table 5.** Percentage degradation ($\Delta T_i = (T_i-T_0)/T_0$) of *L. salivarius* spp. *salivarius* during *in vitro*
586 digestion process over ten days. Values expressed as mean ± standard deviation.
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		Day 1	Day 3	Day 7	Day 10
Encapsulated	ΔT_1	7.4 ± 0.2^{a}	9.5 ± 0.9^{a}	-0.8 ± 0.4^{a}	2.6 ± 1.9^{a}
	ΔT_2	24.2 ± 0.03^{d}	20.0 ± 0.7^{b}	14.0 ± 1.0^{c}	$18.3 \pm 0.5^{b,c}$
	ΔT_3	26.7 ± 0.6^{e}	21.6 ± 0.9^{b}	18.4 ± 1.0^{d}	30.8 ± 1.5^{d}
	ΔT_4	53.4 ± 0.5^{g}	44.9 ± 1.0^{c}	42.8 ± 0.9^{f}	48.9 ± 1.6^{e}
Non-encapsulated	ΔT_1	9.4 ± 0.5^{b}	11.8 ± 0.9^{a}	7.8 ± 1.3^{b}	$9.4 \pm 1.5^{a,b}$
	ΔT_2	19.8 ± 1.2^{c}	21.7 ± 0.5^{b}	22.9 ± 0.9^{e}	19.9 ± 1.0^{c}
	ΔT_3	43.6 ± 0.5^{f}	49.4 ± 0.9^{d}	42.1 ± 1.1^{f}	72 ± 2^{f}
	ΔT_4	73.5 ± 3^{h}	74.8 ± 5^{e}	74.0 ± 2^{g}	84.4 ± 3^{g}

Values with different superscript letters in a column are significantly different ($p \leq 0.05$)
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