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Probiotic survival and in vitro digestion of *L. salivarius* spp. *salivarius* encapsulated by high homogenization pressures and incorporated into a fruit matrix

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ABSTRACT

High pressure homogenization allows encapsulating microorganisms. Microencapsulation of probiotic microorganisms may enhance their viability during food processing, storage and gastrointestinal passage. The aim of this work was to evaluate the probiotic survival and in vitro digestion of non-encapsulated and encapsulated *Lactobacillus salivarius* spp. *salivarius* by homogenization at 70 MPa, included into an apple matrix by vacuum impregnation, dried by hot air drying and stored during 30 days. *Lactobacillus salivarius* spp. *salivarius* was encapsulated with alginate as a coating by homogenization pressures at 70 MPa and it was added to mandarin juice. Juices with L. *salivarius* spp. *salivarius* encapsulated and non-encapsulated were used as impregnation liquid to incorporate the probiotic microorganisms in apple discs. Impregnated apple discs were dried at 40 °C during 24 h and water activity, moisture, counts of viable cells and survival during gastrointestinal simulation for the storage period of 30 days were evaluated. Dried apple discs with encapsulated *L. salivarius* spp. *salivarius* resulted with higher amount of viable cells than in those non-encapsulated. Gastrointestinal simulation results evidenced a protection of the microorganism due to the capsule effect.

1. Introduction

The benefits of probiotic microorganism consumption are increasingly known as scientific evidences demonstrate more and more that probiotic can protect host against a broad range of diseases from infection to psychological and even degenerative diseases (Anderson, Milles, & Tierney, 2017; Avershina et al., 2017; Pirbaglou et al., 2016).

In the development of functional foods with probiotic microorganisms, formulation, processing and storage should favour microorganism survival. Both, technologies and food matrix must be aimed at protecting microorganism cells against external stress factors. In addition, once the food is consumed, the effect of digestion through the gastrointestinal system must be taken into account.

The inclusion of probiotic microorganisms into the structure of a food matrix can help to maintain the integrity of the microbial cells. Moreover, hot air drying technology permits increasing the product shelf life by reducing the water activity and therefore the development of pathogenic microorganism and conferring specific characteristics (Betoret, Betoret, Rocculi, & Dalla Rosa, 2015). Under heat treatment conditions there is a loss of probiotic viability and a stress response is activated which mechanism are under study (Capozzi, Arena, Russo, Spano, & Fiocco, 2016; Fiocco et al., 2010; De Angelis & Gobbetti, 2004). Afterwards, in a dehydrated regime, the probiotic viability increases while decreasing the water activity (Ubbink & Krueger, 2006).

Microencapsulation of probiotic bacteria can be a very useful strategy to maintain survival rates and viability higher during processing over the shelf life and after consumption when compared to non-encapsulated cells (Burgain, Gaiani, Linder, & Scher, 2011; Capela, Hay, & Shah, 2005). The production of microcapsules containing probiotics falls into three main categories: extrusion, emulsion and spray drying. Spray drying technologies are well established, since the size of microcapsules are reduced (few hundred of microns) and homogeneous (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2012). However, spray drying impart physical stresses to the cells such as heat and also

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increase their exposure to oxygen thus greatly reducing the viability of the sensitive probiotic bacteria (Lee & Heo, 2000). The emulsion method of encapsulation by agitation is considered a more gentle process which can be easily monitored thus more viable cells can survive the encapsulation process (Capela et al., 2005). However, the obtained capsules are bigger and less homogeneous than in the other technologies. In order to minimize these disadvantages, high-pressure vale homogenizers can create small droplets by forcing liquids through a narrow valve under pressure. Homogenizers are already a well stablished technology in some food industries such as milk or fruit juices and can operate in continuous thus being not expensive and facilitating the up-scaling (Calabuig-Jiménez et al., 2019; Ding & Shah, 2009).

In 2009, Ding and Shah applied 70 or 138 MPa for the encapsulation of *L. salivarius* starting from an emulsion of sodium alginate and vegetable oil. These process conditions gave microcapsules having a diameter 85–66 μm with an encapsulation yield of 77% (Ding & Shah, 2009). Patrignani et al. studied in 2017 the application of 50 MPa to encapsulate *L. salivarius*, using sodium alginate in emulsion with vegetable oil. Authors obtained an encapsulation yield of 87–83% and the diameters of the capsule obtained, sphere like and quite rough were < 100 μm (Patrignani et al., 2017). Tolerance to high pressure vary according to the species, strain and suspending mediums used (Abee & Wouters, 1999) but generally, the application of pressure under 100 MPa was not able to induce stresses to the microbial cells (Burns et al., 2015; Lanciotti, Patrignani, Iucci, Saracino, & Guerzoni, 2007) and cell death occurred in the range 130–800 MPa (De Angelis & Gobbetti, 2004).

The aim of this work was to determine the probiotic survival and in vitro digestion of *Lactobacillus salivarius* spp. *salivarius* encapsulated by homogenization pressures, included into an apple matrix by vacuum impregnation, dried by hot air drying and stored during 30 days.

2. Material and methods

2.1. Strain and food materials

The strain used in this study was *Lactobacillus salivarius* spp. *salivarius* CECT 4063 provided by the Spanish Type Culture Collection (CECT, Valencia, Spain) in lyophilized form.

Juice was obtained from mandarin fruits cv. Ortanique (*Citrus sinensis* x *Citrus reticulata*) provided by a local cooperative (Rural S. Vicent Ferrer, Benaguacil, Valencia, Spain). Low pulp juice was prepared following the procedure described in WO/2007/042593 with some modifications (Calabuig-Jiménez et al., 2019).

Apples (cv. *Granny Smith*) were purchased from a local market. In this experimental study apple discs with 5 mm thick and 20–60 mm of internal and external diameter were used.

2.2. Microencapsulation

To microencapsulate L. *salivarius* spp. *salivarius* the method described by (Ding & Shah, 2009) was followed with some modifications (Calabuig-Jiménez et al., 2019). Briefly, an emulsion containing 25 mL of microorganism with 10^9 CFU/mL, 100 mL of sodium alginate (3%) (Sigma-aldrich, Steinheim, Germany), 1 mL of tween 80 (Sharlau, Sentmenat, Spain) and 200 mL of sunflower oil was homogenized in two passes at 70 MPa with a homogenizer (Panda Plus Niro Soavi, Parma, Italy). After homogenization calcium chloride 0.1 M (Sigma-aldrich, Steinheim, Germany) was added and microcapsules were isolated by centrifugation at 7700 × *g* for 15 min at 10 °C (Beckman Coulter AvantiTM J-25, California, United States).

2.3. Mandarin juice with probiotic microorganisms

Mandarin juices with *L. salivarius* spp. *salivarius* encapsulated and not were used as impregnation liquids. Mandarin juice with non-encapsulated *L. salivarius* spp. *salivarius* was prepared following the methodology described in (Betoret, Calabuig-Jimenez, Patrignani, Lanciotti, & Dalla Rosa, 2017) following inoculation with 10^9 CFU/mL and incubation at 37 °C for 24 h. 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) (Calabuig-Jiménez et al., 2019). The mixture was maintained in agitation at room temperature for 1 h.

2.4. Process to produce L. salivarius spp. salivarius enriched dried apple

Dried apple discs with *L. salivarius* spp. *salivarius* encapsulated and not, were obtained following the methodology described previously (Betoret et al., 2012). A vacuum pressure of 50 mbar for 10 min was applied to immersed fresh apple discs following an atmospheric pressure restoration during further 10 min. Impregnated apple discs were dried using an air drier (POL-EKO model CLW400 TOP, Controltecnica Instrumentación Científica, S.L., Madrid, Spain) at 40 °C for 24 h. The values provided are the average of three replicates.

2.5. Physicochemical characterization

Impregnated and dried apple discs were characterized by measuring pH, water activity and moisture content. To determine pH, a pHmeter (Crison GLP21, Barcelona, Spain) was used. Water activity was measured using a dew point hygrometer (DECAGÓN Aqualab CX-2, Washington, United States). Water content was quantified by vacuum drying at 60 °C until a constant weight. The values provided are the average of three replicates.

2.6. Microbial content

L. salivarius spp. *salivarius* was determined in MRS agar (Scharlab, Barcelona, Spain) on double layer incubated 24 h at 37 °C. In encapsulated samples the first dilution was done in phosphate buffer solution stirred during 30 min. Values provided are the average of four replicates.

2.7. Gastrointestinal digestion

The effect of the gastrointestinal digestion on the microorganism survival was determined following the procedure described in (Calabuig-Jiménez et al., 2019). T_i was the *L. salivarius* spp. *salivarius* content; t_i was a moment during the gastrointestinal digestion. Briefly, 10 g of sample were mixed with 10 mL of pepsine (0.6% w/v) (Sigma-aldrich, Steinheim, Germany) adjusted to pH 3 with HCl 4 M (t_1 - T_1) and mixed at 37 °C for 90 min (t_2 - T_2). Phosphate buffer solution (pH 8) with 10% of bile (Sigma-aldrich, Steinheim, Germany) was added (t_3 - T_3). Phosphate buffer with 0.3% of bile and 0.1% pancreatine (Sigma-aldrich, Steinheim, Germany) was added following an incubation at 37 °C for 90 min (t_4 - T_4). The results provided are the average of four replicates.

2.8. Storage

Dried samples were stored in closed opaque plastic bags at room temperature and analyses were performed weekly during 30 days.

2.9. Statistical analysis

The significant effect of the process variables, at 95% confidence level, was determined with an ANOVA analysis using Statgraphics centurion XVI software (StatPoint Technologies, Virginia, US).

Table 1
Physicochemical properties of the dried apple with encapsulated and non-encapsulated Lactobacillus salivarius spp. salivarius during the storage time. Mean ± standard deviation of three replicates.

	pH		a_w		Moisture (kg_{water}/kg_{dried})	
Day	Encapsulated	Non-encapsulated	Encapsulated	Non-encapsulated	Encapsulated	Non-encapsulated
1	3.44 ± 0.05^{ab}	3.21 ± 0.05^{a}	0.516 ± 0.002^{c}	0.516 ± 0.002^{c}	0.107 ± 0.002^{a}	0.128 ± 0.006^{ab}
7	3.48 ± 0.03^{abc}	3.16 ± 0.08^{a}	0.487 ± 0.006^{a}	0.516 ± 0.002^{c}	0.128 ± 0.012^{b}	0.124 ± 0.006^{ab}
14	3.39 ± 0.09^{a}	3.36 ± 0.08^{b}	0.534 ± 0.002^{d}	0.500~~3~~ $\pm 0.002^{a}$	0.125 ± 0.003^{b}	0.117 ± 0.003^{a}
21	3.55 ± 0.12^{bc}	3.43 ± 0.04^{b}	0.544 ± 0.002^{e}	0.512 ± 0.002^{b}	0.129 ± 0.006^{b}	0.12 ± 0.06^{a}
30	3.6 ± 0.02^{c}	3.6 ± 0.02^{c}	0.505 ± 0.002^{b}	0.533 ± 0.003^{d}	0.132 ± 0.006^{c}	0.136 ± 0.003^{b}

^{abc} ... Values with different superscript letters within the same column are significantly different ($p \leq 0.05$).

3. Results and discussion

3.1. Physicochemical characterization

Physicochemical characteristics of the impregnated and dried apple discs with *L. salivarius* spp. *salivarius* were evaluated during 30 days of storage (Table 1). Generally, the physicochemical properties of dried apple with *L. salivarius* spp. *salivarius* encapsulated and not, were maintained similar during all the storage time. The pH values of dried apple with encapsulated *L. salivarius* spp. *salivarius* were higher, showing less variability than that obtained in samples with non-encapsulated microorganisms. Samples with encapsulated *L. salivarius* spp. *salivarius* had less amount of mandarin juice impregnated than those samples with non-encapsulated microorganisms. Additionally, the encapsulation process could decrease the activity of *L. salivarius* spp. *salivarius* resulting in a lower fermentation activity of the microencapsulated cells which would produce less acidic compounds (Bilenler, Karabulut, & Candogan, 2017; Ribeiro et al., 2014). At the end of the storage there were not differences between both samples.

The rate of food reactions and spoilage microorganisms activity is reduced with lower moisture content, being retarded and even inhibited with a water activity as or below 0.3 (Smith, 2008, pp. 116–117). In our case, despite obtained water activity was higher than 0.3, moulds or harmful bacteria were not developed during the storage. Our results were similar to that obtained previously by (Betoret et al., 2012). Water activity values ranged between 0.48 and 0.54 in both cases, with more variability observed in samples with encapsulated *L. salivarius* spp. *salivarius* and a tendency to increase with storage time. In samples with non-encapsulated *L. salivarius* spp. *salivarius,* the values of water activity were maintained practically constant during 21 days from which had a tendency to increase. The same behaviour was observed for moisture content values. The presence of oil coming from the emulsion to encapsulate *L. salivarius* spp. *salivarius* in the apple slices could difficult the water flux during drying, resulting in a less homogeneous product. An unequal distribution of water content during drying could cause further water migrations during storage, explaining then the differences observed between both samples.

3.2. Effect of technological operations on probiotic survival

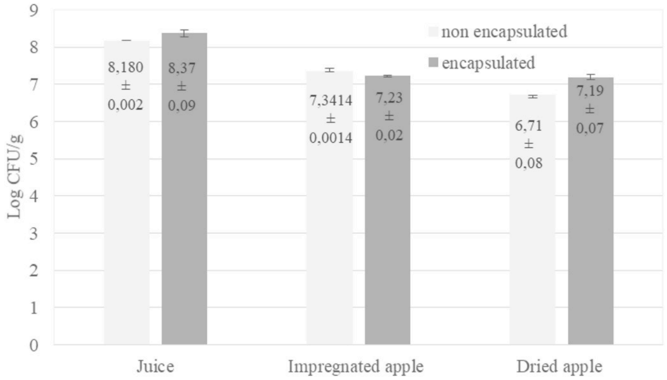

Fig. 1. Microorganism content expressed in Log CFU/g with encapsulated and non-encapsulated L. salivarius spp. salivarius. Plotted results are the average ± standard deviation of four replicates.

Microbial content of the encapsulated and non-encapsulated *L. salivarius* spp. *salivarius* in the mandarin juice, in the impregnated apple and in the impregnated and dried apple are shown in Fig. 1. The content of encapsulated *L. salivarius* spp. *salivarius* in mandarin juice was managed to be the same as that obtained in samples with non-encapsulated microorganisms in order to compare its degradation during the processing. The obtained results were similar to that obtained in previous studies (Calabuig-Jiménez et al., 2019; Betoret et al., 2012, 2017). The amount of mandarin juice with *L. salivarius* spp. *salivarius* encapsulated and not, incorporated into the apple, using vacuum impregnation, was estimated by mass balances using equation (1). Calculated and experimental obtained values were 8.71 ± 0.02 Log CFU/g_{IV} - 7.23 ± 0.02 Log CFU/g_{IV} and 7.62 ± 0.04 Log CFU/g_{IV} - 7.3414 ± 0.0014 Log CFU/g_{IV} in samples with encapsulated *L. salivarius* spp. *salivarius* and not, respectively. Similar calculated and experimental values, as in samples with non-encapsulated *L. salivarius* spp. *salivarius,* indicated that the liquid flux into the intracellular pores of apple was homogeneous and only due to pressure gradients. A homogeneous vacuum impregnation means that all components of the mandarin juice were incorporated equally. Pressure levels applied during the vacuum impregnation operation in this study do not affect significantly microorganisms (Betoret et al., 2012). Thus, the differences observed between calculated and experimental values in samples with encapsulated *L. salivarius* spp. *salivarius* indicated that the vacuum impregnation operation was not homogeneous and this could be due to three possible reasons: a not homogeneous distribution of the encapsulated microorganisms, an accumulation of microorganism in some areas of the apple structure were the pore sizes are smaller than the capsules, an irregular flows of juice through the structure due to local pressure gradients.

$$x_{aIV} = (x_{mJ} \cdot X \cdot (\rho_{mJ}/\ \rho_{fa}))/(1 + X\cdot(\rho_{mJ}/\ \rho_{fa})) \quad (1)$$

Where:

x; microorganism content (CFU/g or CFU/ml)
X; incorporated liquid (cm^3/cm^3_{sample})
ρ; density (g/cm^3)
aIV; impregnated apple
fa; fresh apple
mJ; mandarin juice

The content of *L. salivarius* spp. *salivarius* encapsulated and not in dried apple samples was significantly different and high enough to have a potential probiotic effect (International Dairy Federation (IDF/FIL), 1992). In order to calculate the degradation of microorganism during drying it is necessary that quantities of microorganisms are expressed in the same basis. Thus, the total degradation of *L. salivarius* spp. *salivarius* encapsulated and not during air drying operation was 6.20–6.38 Log CFU/gIV respectively. Considering the initial values of microorganisms, the degradation of *L. salivarius* spp. *salivarius* encapsulated and not during air drying operation was 0.85 and 0.87 respectively. Heat damage, water losses linked to structural changes and oxidation reactions due to the air exposure affect both cellular plant tissues and microbial cells. Excessive heat unfolds the higher order structure of macromolecules such as protein and nucleic acid, breaks the linkage between monomeric units and eventually causes the destruction of the monomeric units (Corcoran, Stanton, Fitzgerald, & Ross, 2008; Santivarangkna, Kulozik, & Foerst, 2008). Water losses linked to structural modifications and oxidation reactions mainly affects the cytoplasmic membrane of microbial cells by changing its fluidity or the physical state as well as causing lipid peroxidation (Crowe, Hoekstra, & Crowe, 1992). Cells entrapped within the droplets formed by alginate would obtain additional protection by the capsule. However, as according to (Fu & Chen, 2011), the protection of cell viability during drying given by this type of microencapsulation is quite limited. In this study, a mild drying was employed, with an air temperature of 40 °C in order to limit drying stress in bacterial cells but more oxidation reactions could be promoted due to the long air exposure time.

3.3. *Probiotic content during storage time*

The content of *L. salivarius* spp. *salivarius* encapsulated and not, stored during 30 days at room temperature and maintained in closed opaque plastic bags, was determined (Table 2). During the first 14 days of storage a decrease in 60% of the microorganism content was observed. This results agree with (Moumita et al., 2017; Weinbreck, Bodnár, & Marco, 2010) that observed a decrease of 3–5 log in the microorganism content encapsulated and not, after 14 days of storage. From this point, significant differences were observed between both samples, with an improvement in the microorganism survival in encapsulated samples of 39 versus 19% of non-encapsulated at the end of storage.

During storage, cell survival is particularly affected when the food matrix has an elevated water activity ($aw > 0.25$) (Teixeira, Castro, Malcata, & Kirby, 1995). Storage temperature and the presence of atmospheric oxygen might also contribute to reductions in viable cell amounts (Anal & Singh, 2007). Our results, showed up that capsules were not able to protect significantly *L. salivarius* spp. *salivarius* from degradation reactions during the first 14 days of storage. As pointed out by (Dianawati & Shah, 2011) alginate is a porous material that is not able to isolate encapsulated microorganisms from water migrations. According to (Crittenden, Weerakkody, Sanguansri, & Augustin, 2006) presence of atmospheric oxygen was not a significant factor in the microorganisms degradation encapsulated in alginate and maintained at room temperature during storage. However, after 14 days of storage, capsules were able to protect *L. salivarius* spp. *salivarius* from degradation reactions.

3.4. *Gastrointestinal simulation*

In order to exert a positive effect on the host, probiotic microorganisms should maintain their active form during digestion process, being able to survive the action of lytic enzyme and adverse pH until reaching the target point. Moreover, in the case of encapsulated microorganisms the capsule must be a protection from adverse conditions but should release them at the appropriate time and place in the organism. The microbial content after each simulated gastro-intestinal digestion step is shown in Table 3. T_0 is the *L. salivarius* spp. *salivarius* content in dried apple. T_1 and T_2 means the microorganism content after simulated stomach conditions, acid pH change and peristaltic movements respectively. T_3 and T_4 are the microorganism content after the duodenal shock and intestinal juice mixing.

L. salivarius spp. *salivarius* demonstrated to have a potential effect against *Helicobacter pylori* infection. Thus, microorganism survival at gastroduodenal stage, in order to have a potential effect against *H. pylori,* and survival at intestinal step, in order to have a potential probiotic effect, are both key points to consider.

The statistical analysis revealed that all variables studied; the encapsulation procedure, the stage at the simulated gastrointestinal digestion and the storage time had a significant effect ($p \le 0.05$) on *L. salivarius* spp. *salivarius* survival. Generally, encapsulated *L. salivarius* spp. *salivarius* demonstrated higher resistance to gastrointestinal simulation as compared to their free form. Total microorganisms content and survival percentage of encapsulated *L. salivarius* spp. *salivarius* was higher than non-encapsulated one. Degradation tendency of the microorganisms encapsulated and not was different at each stage of the simulated gastrointestinal process as well as during the storage. Obtained results were similar to that obtained in other studies (Ribeiro et al., 2014; Yonekura, Sun, Soukoulis, & Fisk, 2014). Survival of encapsulated *L. salivarius* spp. *salivarius* was mainly affected by the acidic environment created at t_1 and the addition of bile at t_3. Moreover, survival of microorganisms decreased with storage time at gastrointestinal stages t_2, t_3 and t_4 but not at t_1 at which survival percentage remained practically constant. The results obtained in literature on the protective effect of alginate capsules against acidic environmental conditions are contradictory. While in some cases, the capsule created protects the microorganisms against acidic conditions (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2011; Ding & Shah, 2009) in others capsule it does not provide any additional protection (Hansen, Allan-Wojtas, Jin, & Paulson, 2002). As explained by (Cook et al., 2012) it seems that the method used to make the capsule significantly influences the final result. In our case, the capsule conferred a limited protection. A porous capsule surface and its degradation during storage could explain the observed decrease in the *L. salivarius* spp. *salivarius* survival with storage time. Non-encapsulated *L. salivarius* spp. *salivarius* was affected by the acidic environment created at t_1 and the addition of lytic enzymes at t_4. In this case, survival of microorganisms decreased with storage time mainly at t_3.

It is remarkable the increase in microorganisms content observed at day 14 in encapsulated *L. salivarius* spp. *salivarius* and not, and at day 21 in non-encapsulated *L. salivarius* spp. *salivarius.* As pointed out by (Santivarangkna et al., 2008) upon sudden changes in temperature, osmotic pressure or pH, a microbial cell is able to adapt itself to the new environment by adjusting the metabolic flow and genetic expression. After the acidic stress conditions created around cells at pH 3.5 Jin et al. (2012) observed a significant increase in the acid tolerance response mechanism which would promote their growth when optimal conditions are restored.

Table 2

Microbial count (Log CFU/g_{dried}) of encapsulated and non-encapsulated dried apple during the storage time. Number in brackets indicates the survival in percentage respect the first day. Mean ± standard deviation of four replicates.

	Day 1	Day 7	Day 14	Day 21	Day 30
Encapsulated	7.19 ± 0.07^a (100)	5.85 ± 0.12^a (81.3 ± 1.7)	3.03 ± 0.06^a (42.2 ± 0.9)	2.94 ± 0.03^a (40.9 ± 0.5)	2.78 ± 0.14^a (39 ± 2)
Non-encapsulated	6.71 ± 0.08^b (100)	5.26 ± 0.09^b (78.2 ± 1.4)	2.89 ± 0.09^b (43.1 ± 1.4)	2.37 ± 0.05^b (35.4 ± 0.7)	1.3 ± 0.2^b (19 ± 3)

abc … Values with different superscript letters within the same column are significantly different ($p \le 0.05$).

Table 3

Microbial content (Log CFU/g_{dried}) of encapsulated and non-encapsulated dried apple with L. salivarius at the beginning (To) and at each phase of the gastrointestinal simulation process (T_1 to T_4) and over the storage time. Number in brackets indicates the survival in percentage respect the initial content. Mean ± standard deviation of four replicates.

		Day 0	Day 7	Day 14	Day 21	Day 30
Encapsulated	$\mathbf{T_0}$	$7.19 \pm 0.07_{B}^{h}$ (100)	$5.85 \pm 0.12_{B}^{g}$ (100)	$3.03 \pm 0.06_{B}^{cd}$ (100)	$2.94 \pm 0.03_{B}^{f}$ (100)	$2.83 \pm 0.14_{B}^{f}$ (100)
	$\mathbf{T_1}$	$6.03 \pm 0.09_{B}^{f}$ (83.7 ± 0.8)	$5.58 \pm 0.02_{B}^{f}$ (96 ± 2)	$3.71 \pm 0.07_{A}^{g}$ (122 ± 2)	$2.67 \pm 0.09_{B}^{e}$ (90.7 ± 3)	$2.38 \pm 0.09_{B}^{ef}$ (85.6 ± 1.3)
	$\mathbf{T_2}$	$5.81 \pm 0.07_{B}^{e}$ (80.8 ± 0.4)	$5.44 \pm 0.06_{B}^{ef}$ (94 ± 3)	$3.84 \pm 0.04_{B}^{h}$ (127 ± 2)	$2.32 \pm 0.13_{A}^{c}$ (79 ± 4)	$2.0 \pm 0.2_{B}^{e}$ (70 ± 2)
	$\mathbf{T_3}$	$5.26 \pm 0.02_{B}^{d}$ (73.2 ± 0.4)	$3.99 \pm 0.07_{B}^{c}$ (68 ± 2)	$2.96 \pm 0.06_{A}^{bc}$ (97.7 ± 0.9)	$2.04 \pm 0.12_{B}^{b}$ (69 ± 3)	$0.8 \pm 0.3_{A}^{ab}$ (29 ± 9)
	$\mathbf{T_4}$	$5.2 \pm 0.2_{B}^{d}$ (72 ± 2)	$4.20 \pm 0.04_{B}^{c}$ (72 ± 2)	$3.09 \pm 0.12_{B}^{d,e}$ (102 ± 3)	$1.41 \pm 0.13_{A}^{a}$ (48 ± 4)	$0.87 \pm 0.19_{B}^{abc}$ (31 ± 6)
Non-encapsulated	$\mathbf{T_0}$	$6.71 \pm 0.08_{A}^{g}$ (100)	$5.26 \pm 0.09_{A}^{e}$ (100)	$2.89 \pm 0.09\ _{A}^{b}$ (100)	$2.37 \pm 0.05\ _{A}^{cd}$ (100)	$1.3 \pm 0.2_{A}^{cd}$ (100)
	$\mathbf{T_1}$	$3.89 \pm 0.08_{A}^{d}$ (58.1 ± 0.4)	$4.5 \pm 0.3_{A}^{d}$ (86 ± 6)	$3.75 \pm 0.06_{B}^{gh}$ (130 ± 4)	$2.39 \pm 0.13_{A}^{de}$ (105 ± 5)	$1.0 \pm 0.7_{A}^{bc}$ (77 ± 5)
	$\mathbf{T_2}$	$3.55 \pm 0.06_{A}^{d}$ (52.9 ± 0.3)	$4.5 \pm 0.5_{A}^{d}$ (85 ± 9)	$3.18 \pm 0.03_{A}^{ef}$ (109 ± 3)	$2.40 \pm 0.06\ _{B}^{cd}$ (100 ± 0.8)	$1.8 \pm 0.3_{A}^{de}$ (138 ± 15)
	$\mathbf{T_3}$	$3.96 \pm 0.04_{A}^{c}$ (59.1 ± 0.7)	$2.75 \pm 0.12_{A}^{a}$ (52 ± 2)	$3.25 \pm 0.05_{B}^{f}$ (112 ± 2)	$2.0 \pm 0.2_{A}^{b}$ (86 ± 7)	$0.7 \pm 0.8_{B}^{abc}$ (53 ± 62)
	$\mathbf{T_4}$	$1.9 \pm 0.06_{A}^{a}$ (28 ± 0.6)	$3.48 \pm 0.05_{A}^{b}$ (66.2 ± 0.3)	$2.67 \pm 0.02_{A}^{a}$ (92 ± 2)	$1.46 \pm 0.06_{B}^{a}$ (61 ± 2)	$0.3 \pm 0.3_{A}^{a}$ (22 ± 25)

abc … Values with different superscript letters within the same column are significantly different ($p \leq 0.05$).
ABC … Values with different subscript letters within the same column shows significance of encapsulation factor ($p \leq 0.05$).

4. Conclusion

Incorporation of encapsulated *L. salivarius* spp. *salivarius* using homogenization pressures into an apple structure by vacuum impregnation operation was successfully done. In spite of the microorganisms losses during hot air drying operation, the number of *L. salivarius* spp. *salivarius* in the impregnated and dried apple was enough high to have a potential beneficial effect.

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References

Abee, T., & Wouters, J. A. (1999). *International Journal of Food Microbiology, 50*, 65–91.

Anal, A. K., & Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science & Technology, 18*, 240–251.

Anderson, J. L., Milles, C., & Tierney, A. C. (2017). Effect of probiotics on respiratory, gastrointestinal and nutritional outcomes in patients with cystic fibrosis: A systematic review. *Journal of Cystic Fibrosis, 16*(2), 186–197.

Avershina, E., Rubio, R. C., Lundgård, K., Martinez, G. P., Collado, M. C., Storrø, O., et al. (2017). Effect of probiotics in prevention of atopic dermatitis is dependent on the intrinsic microbiota at early infancy. *The Journal of Allergy and Clinical Immunology, 139*(4), 1399–1402.

Betoret, E., Betoret, N., Rocculi, P., & Dalla Rosa, M. (2015). Strategies to improve food functionality: Structure-property relationships on high pressure homogenization, vacuum impregnation and drying operations. *Trends in Food Science & Technology, 46*, 1–12.

Betoret, E., Calabuig-Jimenez, L., Patrignani, F., Lanciotti, R., & Dalla Rosa, M. (2017). Effect of high pressure processing and trehalose addition on functional properties of Mandarin juice enriched with probiotic microorganisms. *LWT-Food Science and Technology, 85*, 418–422.

Betoret, E., Sentandreu, E., Betoret, N., Codoñer-Franch, P., Valls-Bellés, V., & Fito, P. (2012). Technological development and functional properties of an apple snack rich in flavonoid from Mandarin juice. *Innovative Food Science & Emerging Technologies, 16*, 298–304.

Bilenler, T., Karabulut, I., & Candogan, K. (2017). Effects of encapsulated starter cultures on microbial and physicochemical properties of traditionally produced and heat treated sausages (sucuks). *LWT-Food Science and Technology, 75*, 425–433.

Burgain, J., Gaiani, C., Linder, M., & Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of Food Engineering, 104*, 467–483.

Burns, P. G., Patrignani, F., Tabanelli, G., Vinderola, G. C., Siroli, L., Reinheimer, J. A., et al. (2015). Potential of high pressure homogenisation on probiotic Caciotta cheese quality and functionality. *Journal of Functional Foods, 13*, 126–136.

Calabuig-Jiménez, L., Betoret, E., Betoret, N., Patrignani, F., Barrera, C., Seguí, L., et al. (2019). High pressures homogenization to microencapsulate L. salivarius spp. salivarius in Mandarin juice. Probiotic survival and in vitro digestion. *Journal of Food Engineering, 240*, 43–48.

Capela, P., Hay, T. K. C., & Shah, N. P. (2005). Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt. *Food Research International, 39*, 203–211.

Capozzi, V., Arena, M. P., Russo, P., Spano, G., & Fiocco, D. (2016). Stressors and food environment: Toward strategies to improve robustness and stress tolerance. *Probiotics, probiotics, prebiotics, and synbiotics* (pp. 245–256). Cambridge, United States: Academic Press (Chapter 16).

Cook, M., Tzortzis, G., Charalampopoulos, D., & Khutoryanskiy, V. V. (2011). Production and evaluation of dry alginate-chitosan microcapsules as an enteric delivery vehicle for probiotic bacteria. *Biomacromolecules, 12*, 2834–2840.

Cook, M., Tzortzis, G., Charalampopoulos, D., & Khutoryanskiy, V. V. (2012). Microencapsulation of probiotics for gastrointestinal delivery. *Journal of Controlled Release, 162*, 56–67.

Corcoran, B. M., Stanton, C., Fitzgerald, G., & Ross, R. P. (2008). Life under stress: The probiotic stress response and how it may be manipulated. *Current Pharmaceutical Design, 14*(14), 1382–1399.

Crittenden, R., Weerakkody, R., Sanguansri, L., & Augustin, M. A. (2006). Synbiotic microcapsules that enhance microbial viability during nonrefrigerated storage and gastrointestinal transit. *Applied and Environmental Microbiology, 72*(3), 2280–2282. https://doi.org/10.1128/AEM.72.3.2280–2282.2006.

Crowe, J. H., Hoekstra, F. A., & Crowe, L. M. (1992). Anhydrobiosis. *Annual Review of Physiology, 54*(1), 579–599.

De Angelis, M., & Gobbetti, M. (2004). Environmental stress response in Lactobacillus: A review. *Proteomics, 4*, 106–122.

Dianawati, D., & Shah, N. P. (2011). Enzyme stability of microencapsulated *Bifidobacterium animalis* ssp. *lactis* Bb12 after freeze drying and during storage in low water activity at room temperature. *Journal of Food Science, 76*(6), M463–M471.

Ding, W. K., & Shah, N. P. (2009). Effect of homogenization techniques on reducing the size of microcapsules and the survival of probiotic bacteria therein. *Journal of Food Science, 74*(6), M231–M236.

Fiocco, D., Capozzi, V., Collins, M., Gallone, A., Hols, P., Guzzo, J., et al. (2010). Characterization of the CtsR stress response regulon in Lactobacillus plantarum. *Journal of Bacteriology, 192*, 896–900.

Fu, N., & Chen, X. D. (2011). Towards a maximal cell survival in convective thermal drying processes. *Food Research International, 44*(5), 1127–1149.

Hansen, L. T., Allan-Wojtas, P. M., Jin, Y. L., & Paulson, A. T. (2002). Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastro-intestinal conditions. *Food Microbiology, 19*, 35–45.

International Dairy Federation (IDF/FIL) (1992). Physiological and functional properties of probiotics. *Bulletin of the International Dairy Federation, 272*, 17–22.

Jin, J., Zhang, B., Guo, H., Cui, J., Jiang, L., Song, S., et al. (2012). Mechanism analysis of acid tolerance response of Bifidobacterium longum subsp. longum BBMN 68 by gene expression profile using RNA-sequencing. *PLoS One, 7*(12), e50777.

Lanciotti, R., Patrignani, F., Iucci, L., Saracino, P., & Guerzoni, M. E. (2007). Potential of high pressure homogenization in the control and enhancement of proteolytic and fermentative activities of some Lactobacillus species. *Food Chemistry, 102*, 542–550.

Lee, K. Y., & Heo, T. R. (2000). Survival of Bifidobacterium longum immobilized in calcium alginate beads in simulated gastric juices and bile salt solution. *Applied and Environmental Microbiology, 66*, 869–873.

Moumita, S., Goderska, K., Johnson, E. M., Das, B., Indira, D., Yadav, R., et al. (2017). Evaluation of the viability of free and encapsulated lactic acid bacteria using in-vitro gastro intestinal model and survivability studies of synbiotic microcapsules in dry food matrix during storage. *LWT-Food Science and Technology, 77*, 460–467.

Patrignani, F., Siroli, L., Serrazanetti, D. I., Braschi, G., Betoret, E., Reinheimer, J. A., et al. (2017). Microencapsulation of functional strains by high pressure homogenization for a potential use in fermented milk. *Food Research International, 97*, 250–257.

Pirbaglou, M., Katz, J., de Souza, R. J., Stearns, J. C., Motamed, M., & Ritvo, P. (2016). Probiotic supplementation can positively affect anxiety and depressive symptoms: A systematic review of randomized controlled trials. *Nutrition Research, 36*(9), 889–898.

Ribeiro, M. C. E., Chaves, K. S., Gebara, C., Infante, F. N., Grosso, C. R., & Gigante, M. L. (2014). Effect of microencapsulation of Lactobacillus acidophilus LA-5 on physicochemical, sensory and microbiological characteristics of stirred probiotic yoghurt. *Food Research International, 66*, 424–431.

Santivarangkna, C., Kulozik, U., & Foerst, P. (2008). Inactivation mechanisms of lactic acid starter cultures preserved by drying processes. *Journal of Applied Microbiology, 105*(1), 1–13.

Smith, P. G. (2008). *Applications of fluidization to food processing introduction.* Wiley-Blackwell.

Teixeira, P. C., Castro, M. H., Malcata, F. X., & Kirby, R. M. (1995). Survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* following spray-drying. *Journal of Dairy Science, 78*(5), 1025–1031.

Ubbink, J., & Krueger, J. (2006). Physical approaches for the delivery of active ingredients in foods. *Trends in Food Science & Technology, 17*, 244–254.

Weinbreck, F., Bodnár, I., & Marco, M. L. (2010). Can encapsulation lengthen the shelf-life of probiotic bacteria in dry products? *International Journal of Food Microbiology, 136*(3), 364–367.

Yonekura, L., Sun, H., Soukoulis, C., & Fisk, I. (2014). Microencapsulation of *Lactobacillus acidophilus NCIMB* 701748 in matrices containing soluble fibre by spray drying: Technological characterization, storage stability and survival after *in vitro* digestion. *Journal of Functional Foods, 6*, 205–214.