VIABILITY OF SOME PROBIOTIC COATINGS IN BREAD AND ITS EFFECT ON THE CRUST MECHANICAL PROPERTIES

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Abstract

The objective of this study was to obtain functional bread combining the microencapsulation of *Lactobacillus acidophilus* and starch based coatings. Different probiotic coatings (dispersed or multilayer) were applied onto the surface of partially baked breads. In all treatments, microencapsulated *Lactobacillus acidophilus* survived after baking and storage time, although reduction was higher in the sandwich treatment (starch solution/sprayed microcapsules/starch solution). Despite coatings significantly affected the physicochemical properties of the crust, increasing water activity and reducing the failure force, the sensory evaluation revealed a good acceptability of the functional breads. Scanning electron microscopy revealed the presence of scattered microcapsules onto the bread crust, being highly covered in the sandwich coating. Therefore, *Lactobacillus acidophilus* included in microcapsules can be incorporated to bread surface through edible coatings, leading functional bread with similar characteristics to common bread, but with additional healthy benefits.

Key words: starch; probiotic coatings; bread; microstructure; crust.

1. **Introduction**

Bread is staple food in many countries, since it constitutes an important source of complex carbohydrates, proteins, minerals and vitamins (Rosell, 2007). In recent years, consumers' interest in the role of nutrition for health and wellbeing has increased. Therefore, today, the priority of the industry is to innovate, meet and satisfy consumer requirements. Concerning baking industry, that trend has prompted the development of baked goods keeping in mind the healthy concept. All the whole meal products or the fiber enriched baked goods would fall in this category (Redgwell and Fischer, 2005). However, functional breads containing viable microorganisms have not been developed yet due to the high temperature reached during baking.

Guarner and Schaafsma (1998) defined probiotic as a live microorganism, which upon ingestion in certain numbers, exerts health benefits beyond inherent basic nutrition. Various species of genera *Lactobacillus* and *Bifidobacterium* have been used as probiotics (Lian et al., 2002; Lavermicocca et al., 2005). Sometimes survival of many probiotic bacteria during processing and storage is insufficient and limits its usefulness in food applications. Therefore, alternative for providing viable microorganisms are the microencapsulation technique. The encapsulation protects probiotic from environmental and physiological degradation (Lian et al., 2002; Capela, Hay and Shah, 2006).

Edible coatings are materials which can be consumed and provides a barrier to moisture, oxygen and solute movement for the food. Edible coatings are particular forms of films directly used onto the surface of materials, which become an element of the ending product (Cuq et al., 1995). This type of coatings is prepared with biological materials such as proteins, lipids and polysaccharides (Tharanathan, 2003). However, starch is one of the preferred types of coatings because it is abundant, cheap and biodegradable. Moreover, the

incorporation of functional ingredients on the edible coatings would be an alternative for protecting the microorganisms. In bakery products, microencapsulation has been extensively used for protecting iron salts and increasing its bioavailability (Cocato et al., 2007). Partially baked bread is an alternative product that shows an expansion trend, owing to provide fresh bread available all time of day (Rosell, 2009). This bread only requires a short baking for obtaining full baked bread. Therefore, it constitutes a potential food for obtaining functional bread combining the microencapsulation and coating technologies.

The objective of this study was to determine the viability of different types of functional coatings applied onto the surface of partially baked breads before full baking step. The survival of microorganisms (*Lactobacillus acidophilus*) was assessed after baking and after a short storage (24 hours). Fresh breads were sensory evaluated and the physical and chemical properties of bread crust were determined. Special attention has been paid to the coatings and bread crust microstructures.

2. Materials and methods

The strain of *Lactobacillus acidophilus* used for microencapsulation was obtained from Danisco Ingredients México, S.A. (México). Whey protein isolates (WPI) from Davisco Foods International Inc. (EUA), carboxymethylcellulose (CMC, Aqualon® Cellulose gum, 7LF PH) from Hércules Incorporated (EUA), low methoxyl citric pectin (P) (Grindsted, Pectin RS 400) from Danisco Méxicana, S. A. (México), inulin (I) from Quantum Natura S. A. (México), fresh agave sap without fermentation (aguamiel) from San Juan de las Manzanas (México) were used for encapsulation. Commercially available corn starch (Maizena) from Unilever Food solutions (México) wasusedfor coating. Some corn starch

characteristics are as follows: maximum viscosity during heating 1847cP, viscosity at 50ºC 1531cP, gelatinization peak temperature 66ºC, gelatinization enthalpy 10 J/g.

A partially baked bread specialty available in the Spanish market was used. Part-baked frozen breads were provided by Forns Valencians S.A. (Valencia, Spain) and stored at -18° C until use. The qualitative composition of the breads includes breadmaking wheat flour, water, yeast, salt and bread improver. Its chemical proximate composition was: 30.1% moisture content, 60% carbohydrates, 6.41% proteins and 2.74 % fats.

2.1 Microbial encapsulation

Microcapsules formation was based on previous results (Rodríguez-Huezo et al., 2007; Villa-García et al., 2010). Briefly, encapsulating agents were prepared by dispersing whey protein isolates, CMC, pectin, inulin and fresh agave sap in a proportion of 45.67 : 11.72 : 18.72 : 22.83 : 1.06., in order to obtain a suspension concentration of 6.57% (w/w).

Lactobacillus acidophilus was used as probiotic. The pure culture of *L. acidophilus* were developed in MRS medium (de Man, Rogosa & Sharp, DIFCO) with low oxygen tension. After growing, they were transferred to 0.1% peptone water.

One litre of watery dispersions of encapsulating agents was combined with the inoculum, previously adjusted to 5 McFarland standard of turbidity (bioMérieux). The mixture was homogenized during for 10 minutes at room temperature and then spray dried by a Niro Atomizer dryer, provided with a rotatory atomizer set on an input/output temperature of 130°C/65°C, with a 2 bar pressure and a feeding of 15 ml/min.

2.2. Edible coating preparation and characterization

2.2.1. Edible coating preparation

Three different treatments were prepared (S1, S2, S3), which differed in the number of coating layers applied onto the bread surface (Table 1). Starch suspension (5%, w/v) was

used as coating material. Preliminary tests were carried out to optimize the level of microcapsules in each treatment in order to have similar microbes' survival after baking. Treatment S1 consisted in the starch solution (5%, w/v) containing microcapsules (1%, w/v). Treatment S2 was as described in S1 plus a coating with starch solution (5%, w/v). In treatments S1 and S2, microcapsules (1%, w/v) were added to the starch suspension and kept under magnetic stirring for ensuring uniform dispersion. Treatment S3 consisted in a coating of starch solution (5%, w/v), followed by dispersing microcapsules (2% w/w, which corresponded to 0.2 g/bread), and a final coating with starch solution (5%, w/v), like a sandwich. Treatment S3 required double microbes' concentration than treatment S1 and S2 to obtain similar survival after subjected to baking.

2.2.2. Coating Properties

Mechanical properties, film thickness and morphology of the coating were determined. Test filmstrips (6 X 2.0 cm) were cut from preconditioned samples (23°C; 75% RH) and mounted between the grips of the probe A/TGT of the TA.XT2i texturometer (Stable Micro Systems, UK). The tests were conducted according to the ASTM D882-00 (2001) method (Veiga-Santos et al., 2005). Ten specimens were tested for each formulation.

Average film thickness of the preconditioned samples (7% RH, 25°C) was obtained using a flat parallel surface micrometer with 1 picometer resolution. Five measurements were taken at three different randomly selected positions.

The coatings were placed on glass plates and the scanning photography was carried out on flatbed scanner (HP Scanjet 4400c). One representative sample (of three) from each of the coatings was chosen for digital documentation.

Cross sections of the starch film and microcapsules powder samples were sprinkled onto double-backed cellophane tape attached to a stub. They were vacuum coated by evaporation with silver and examined by means of a JEOL JSM-5310LV scanning electron microscope (SEM) (JEOL Korea Ltd., Korea) at an accelerating voltage of 12 kV.

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143 **2.3 Full baking process and storage**

144 Part-baked breads were removed from the freezer and thawed at room temperature till the
145 center of the loaf reached 5ºC. Breads were baked off in a forced convection oven
146 (Eurofours, Gommegnies, France) under the following conditions: preheating of the oven
147 at 220° C, and convection during 16 min at 180° C. Then, 10 ml of probiotic coating
148 solution were evenly sprayed over the top surface (118.3± 1cm^2) of the breads before
149 baking. When various coatings were applied (S2 and S3) they were sprayed successively
150 onto the surface of the partially baked bread (Table 1).
151 After bake off, breads were allowed to cool down and stored in a cabinet at 25° C and
152 relative humidity (RH) of 61%. Three sets of loaves were prepared for each treatment and
153 they were baked in separate days.

154

155 2.3.1 Microbiological analysis

156 The amount of viable *Lactobacillus acidophilus* in the bread surface was determined after
157 full baking 0.5 h (fresh bread), and after 24 h storage. A bread crust portion (1g) was
158 aseptically diluted in 9 ml of sterile peptone water solution (Scharlau Chemie, Barcelona,
159 Spain) and mixed for 1 min in Lab Blender 400 Stomacher (Seward Medical, London,
160 UK). Serial dilutions were made in sterile peptone water and plated following the surface
161 technique onto De Man, Rogosa and Sharpe (MRS, Scharlau Chemie, Barcelona, Spain)
162 agar supplemented with 10% sterile skim milk. The culture medium contained a second
163 layer of MRS agar used for generating anaerobic conditions. The agar plates were
164 incubated at 32° C for 5 days. After the respective incubation times, results were recorded
165 as colony-forming units (CFU)/g of product.

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167 2.3.2 Chemical and physical analyses

Analysis of the bread samples was performed at 0.5h (fresh bread), 2, 4, 6, 8 and 24 h after baking. Bread volume was determined by the rapeseed displacement method. Crust colour parameters were measured at three different locations of the surface by using a Minolta colorimeter (Chroma Meter CR-400/410, Konica Minolta, Japan) after standardization with a white calibration plate (L^* = 96.9, a^* = -0.04, b^* = 1.84). The colour was recorded using CIE-L^* a^* b^* uniform colour space (CIE-Lab), where L^* indicates lightness, a^* indicates hue on a green (-) to red (+) axis, and b^* indicates hue on a blue (-) to yellow (+) axis.

Crust moisture content and water activities were followed during short bread storage. Crust was separated using a razor blade. Moisture content was determined according to the ICC Method (110/1, 1994). Water activities were measured using a water activity unit (Aqua Lab Series 3, Decagon devices, Pullman, USA) at 25°C.

All determinations were carried out in triplicate. The results presented are averages of all available replicates.

2.3.3 Puncture tests

Breads were puncture tested at a deformation speed of 40 mm/s using a 4mm diameter cylindrical probe. Experiments were performed using a texture analyzer (TA XTplus, Stable Micro Systems, Surrey, UK). The peak force and the peak deformation point of the crust were calculated by punching the samples at eight different points of bread surface: left and right sides, 2 cm distance from the middle point. The average value was calculated for each sample. The failure force was calculated as the peak force observed according to studies by Jackman and Stanley (1992). The failure deformation, defined as the deformation at the peak point, was also calculated. The failure firmness, defined as the slope of load displacement curve from zero to the point of rupture or failure, was

calculated according to studies by Shafiee et al. (2008). Three bread samples were used for each measurement.

2.3.4. SEM of bread crust

The structure of the treated crusts were analysed by scanning electron microscopy. Freeze-dried samples of the crust were mounted on metal stubs and the samples were coated with a gold and palladium layer (100–200 Å) by Ion Sputter (Bio-Rad SC-500). All samples were examined using an accelerating voltage of 10 kV with a scanning electron microscope (S-4100, Hitachi, Ibaraki, Japan) equipped with a field emission gun, a back-secondary electron detector and an EMIP 3.0 image data acquisition system (Rontec, Normanton, UK) from the SCSIE Department of the University of Valencia.

2.3.5. Sensory evaluation

Sensory evaluation was carried out by a trained panel of eight judges and scored on a scale of 1 (dislike extremely) to 5 (like extremely). Sensory tests were carried out under normal lighting conditions and at room temperature. The experience of the judges in this type of analysis for bread products varied from 3 to 20 years. Preliminary training was performed to evaluate crust appearance, odour, , crust colour, crispness and crumb hardness. For each one of these attributes, the average response was reported.

2.4 Statistical analysis

All data were presented as mean values of at least three replicates. Statistical analysis of the results was performed using Statgraphics Plus V 7.1 (Statistical Graphics Corporation, UK). Data were analyzed by nonparametric one-way analysis of variance (ANOVA).

When ANOVA indicated significant F values, multiple sample comparison was also performed by Tukey HSD test in order to detect significant differences.

3. Results and Discussion

3.1 Probiotic coatings

Lactobacillus acidophilus is a microorganism that requires low oxygen tension, and essential nutrients as carbohydrates, proteins, vitamins of the B-Complex, nucleic acids and minerals. Thus, these nutrients must be available in the medium for growth and establishment of a predominant microflora of lactobacilli (Gomes and Malcata, 1999). Microcapsules composition was selected based on reported results of activation energy (E_a) that is a good parameter for the selection of spray drying encapsulated materials. Higher values of this parameter deliver better microcapsules (Rodríguez-Huezo et al., 2007). Previous findings showed that the mix of protein and agave sap largely increases the activation energy (35.7 kJ/mol) and that the agave sap, as well as the inulin, provides major E_a 37.92 kJ/mol when they are combined (De Jonge et al., 2007; Martínez and Morales, 2007; Villa-García et al., 2010). Moreover, the addition of hydrocolloids like pectin and CMC to the previous mix further increases the E_a (40.3 kJ/mol). It seems that the combination of the inulin and agave sap with the selected hydrocolloids could have some interaction with the lipids of the cell membrane of the microorganisms and help to protect the probiotics during drying in the encapsulation process.

Figure 1 presents the digital images of probiotic coatings. Probiotic coating S1 showed a moderately regular surface and cohesive layer (Figure 1a), while a slightly fragmented and opaque layer was observed in S2 (Figure 1b) resulted from the double layer. When microcapsules were sprayed, as in coating S3, a non cohesive and an uneven as well as fragmented surface was obtained (Figure 1c). Mechanical characterization of probiotic

films showed that S3 had the highest tensile strength (1.86 ± 0.74 kg), followed by S1 (0.69 ± 0.52 kg) and S2 (0.41 ± 0.19 kg), despite no significant differences were detected among the thickness values (77.67±15.37µm, 56.89±10.43µm, 66.78±6.53µm for S1, S2 and S3, respectively) (Figure 2). It should remark that films were prepared without swelling the starch granules, thus no significant change in the film thickness was initially expected. Likely, the incorporation of microcapsules within the film, which occurred in S1 and S2, interrupts the starch based film structure leading to a decrease in the mechanical resistance.

The microstructure analysis (Figure 2) showed two different populations of microcapsules (Figure 2a), one with diameters ranging from 2-5µm and the other with diameter around 20µm. The small population had smooth and corrugated surface, forming like-tube cages surrounding cell agglomerations, and the large microcapsules were more spherical with smooth surface enclosing microcapsules as described before. Apparently *L. acidophilus* protection is complete because free bacteria were not observed. Previous findings suggested that the rough surface gives protection to probiotics (De Jonge et al., 2007; Martínez and Morales, 2007; Villa-García et al., 2010). Probiotic films showed continuous and smooth surface but microcapsules surrounded by a layer of starch were envisaged (Figure 2b). Comparing the cross sections of the three films, it was observed that S1 (Figure 2c) presents a continue network of starch very similar to the one of the film without microcapsules. S2 had denser cross section exhibiting accumulations of microcapsules at the surface (Figure 2d). In addition, film S3 (Figure 2e) showed an interior network of starch, and two layers of higher density at both sides of the film.

3.2 Viability of *Lactobacillus acidophilus* in bread crust

The microbial count was determined after baking and after 24h storage to detect the microbial viability after baking and also its stability during bread storage (Table 2). Viable microorganisms remained after the baking process in all the coatings. Therefore, those coatings could be used for obtaining viable microorganisms containing breads. However, the temperature reached during the full baking process of the partially baked bread affected in different extent depending on the coatings studied. Breads with S2 treatment kept 63.2% of the counts after baking. Considering that breads with treatment S3 had more microcapsules concentration, the treatment S3 was the most sensitive to baking temperature. Quezada-Gallo et al. (2004) showed that when comparing the functional properties of dextrans, starch and four highly purified biopolymers (xanthan gum, sodium alginate, carboxymethylcellulose and tragacanthin) dextrans and starch showed better properties as gas barrier. In addition, when starch-based coating was applied onto white bread and doughnuts, results showed that starch coatings controlled additives liberation to the product as a function of its water activity (Quezada-Gallo et al., 2004). It seems that coatings somewhat protects the microorganisms viability even after the baking process. In addition, the stability of the microorganism during short storage of the breads was investigated. The short-term storage caused a reduction in the total colony counts of microencapsulated *Lactobacillus acidophilus* in all treatments. The reduction in the microbial counts during the storage period was similar in all the treated breads, independently of the coating treatment (Table 2). Therefore, it seems that the immediate surroundings of the microbes are responsible of this result, and only the microcapsules composition, which provides the essential micronutrients, determines the viability of the microbes during storage. Despite the reduction, microbes' survival indicates that probiotic coatings can be applied to the bread crust for obtaining functional breads.

3.3 Effect of probiotic coatings on the physico-chemical properties and sensory evaluation of bread

Loaves used in this study have half-cylindrical geometry of size roughly 15±0.03 x 2.5±0.1 x 3.0±0.1 cm, weight 70±2 g and crust thickness was roughly 2.96±0.45 mm. Breads were sensory evaluated and no difference in taste was detected due to the presence of coatings. All the breads were accepted and no significant differences were observed regarding the attributes scored (Table 3). Perhaps the most affected attribute was the crust colour, namely in S3 due to the presence of some brown spots that corresponded to the microcapsules.

Some physical and chemical properties of bread were studied. The values obtained for crust colour, specific volume, water activity and moisture content are showed in Table 3. No differences were detected in the moisture content of the crumb, neither in the water activity or texture properties of the crumb due to the different coating treatments (results not showed). Unexpectedly, the treatment S2 produced a significant ($p < 0.05$) decrease in the specific volume of bread; whereas, the control and breads with treatments S1 and S3 showed the same specific volume of the bread. Coatings were applied onto the partially baked breads surface, thus it seems that treatment S2 impairs any expansion that could take place during full baking.

The colour of the bread crust was also affected by treatments with probiotic coatings. The loaves with treatment S3 had the lowest lightness *(L*)*, which might be attributed to the spraying of microcapsules between two coatings of starch solution, and the uneven mixing with the coatings produced an increase of opaque colour. Craig et al. (1989), when studied the visual characteristic of aqueous starch paste, observed that during gelatinization the starch granules swell and more light passes through the granules instead of being reflected. Therefore, the ability of the granules to reflect light diminishes, whereas, the transmitted

316 light passing through swollen granules is refracted and the degree of refraction decreases
317 with increasing swelling of the granules.
318 The treatment S1 did not promote any effect on the *a** parameter of the crust with respect
319 to control bread; but the double addition of starch solution in treatment S2 and S3
320 produced a decrease in this parameter. Again, the presence of double starch solution
321 yielded the lowest *b** values of the crust.
322 The water activity and the moisture content of the crust ranged from 0.43 to 0.56, and
323 6.6% to 9.9%, respectively. Coatings significantly (p<0.05) increased the crust water
324 activity. Sample S3 showed the highest water activity, likely due to the hydrophilic
325 structure of the starch based coatings. The moisture content of the crust significantly
326 (p<0.05) increased with treatments S1 and S3. In opposition, treatment S2 showed lower
327 moisture content than the control. It seems that in coating S2 the incorporation of
328 microcapsules on starch solution and the application of a second starch solution over crust
329 bread become more rigid and difficult to disperse, yielding microcapsules accumulation
330 (Figure 2d). Likely a more cracking film was obtained which favored the water diffusion
331 (Müller et al., 2009).
332 The probiotic coatings applied on the bread surface produced significant changes on the
333 mechanical properties of the crust (Table 3). The failure force or force necessary to induce
334 the crust fracture significantly (p<0.05) decreased with the coatings applied. The treatment
335 S2 resulted in the lowest force for fracture, despite its lower water content. Considering
336 that at Aw<0.6 high failure force indicated brittle crust. Goedeken (1993) found that if
337 microstructure is more porous, it gives brittle behavior and eases the water diffusion
338 through the crust, strongly affecting the permeability of porous materials. A crispy texture
339 has been associated with low values of moisture content and water activity, when starch
340 and gluten matrix are in a glassy state and thus cell walls become more susceptible to

fracture (Stokes and Donald, 2000). Therefore, starch based coatings decreased the mechanical properties associated to crispness, but sensory analysis indicated that those changes did not induce significant differences in the perceived attributes.

The failure deformation increased significantly (p<0.05) in the treated breads, with the exception of breads treatment with S1, which presented lowest deformation value than control bread. High values of failure deformation have been obtained in breads with thick crust suggesting stiffer crust structure (Altamirano-Fortoul and Rosell, 2011). Although no significant differences were found in the coatings thickness, the higher failure deformation observed in the crust with double starch coating suggested the presence of thicker crust in those samples, which would be expected after the swelling occurred during full baking.

3.3 Effect of storage conditions

No crust separation was observed during the bread storage, neither in the control or the treated bread. The moisture content of the crust increased with the storage time (Figure 3a), observing the most rapid increase during the first four hours after baking, which agrees with previous results of Altamirano-Fortoul and Rosell (2011). No significant effect was observed in the presence of the different coatings, the only distinguishable effect was observed in S2 that had lower initial slope, but higher moisture content when reaching the plateau. If the initial slope of the plots is taken as a measure of the speed of water uptake by the crust, the coating S2 reduced that speed, which has been related to higher porosity of the bread crust (Primo-Martín et al., 2008). This observation agrees with the coating microstructure of S2 that had accumulation or agglomerates of microcapsules, which could facilitate water diffusion from the crumb and the atmosphere surroundings. However, after prolonged storage high water uptake was obtained likely due to the agglomerates hydration.

Crust water activity was also affected by probiotic coatings during storage time (Figure 3b). This parameter also presented a rapid initial increase during the first four hours after baking. Control crust showed the highest increase of water activity. It has been described that at Aw = 0.6 water content starts to increase in an exponential fashion producing film structural changes which allow a facilitated water transport phenomenon (Bertuzzi et al., 2007). Coatings decreased the slope of the curves, being the highest reduction observed with treatment S2. Considering together the results obtained of treatment S2 for moisture content and water activity, it seems that at short storage period microcapsule agglomerates reduced the ability of the crust to retain water. However, after 4 hours storage agglomerates hydration might be responsible of higher crust moisture content with tightly bound water molecules, as suggest the lower water activity observed.

With respect to the mechanical properties of bread with probiotic coatings, failure force increased throughout the time of storage (Figure 3c). The crust on control bread presented an increase in failure force as a result of moisture migration from the crumb to the crust and from the surrounding atmosphere to the crust. Consequently, the initially crispy crust becomes soft and leathery within very short period of storage. Samples with coatings showed initially lower crust failure force than the control, and the same trend was observed along the storage period. Loaves with treatment S2 presented the lowest value of failure force after 6 and 24 h of storage, thus coating S2 showed small increase of the failure force during storage. Coatings modified the crust structure, due to the new layers addition, which decreased the failure force in the fresh bread, but they did not significantly modify the trend during bread storage.

3.4 Crust structure

391 Scanning electron microscopy observations of surface and cross-section of probiotic crusts
392 are showed in Figure 4. Control crust showed a continuous veil-like film that revealed a
393 dominant presence of the partially gelatinized starch granules (Figure 4a), whereas the
394 cross section showed a compact structure resulting from starch gelatinization and protein
395 denaturation (Figure 4b).
396 Probiotic coatings induced significant differences in the crust microstructure. The crust
397 with coating S1 showed a smooth and homogenous background due to gelatinized starch,
398 together with some agglomerates of roughly polyhedral microcapsules (Figure 4c). The
399 micrograph of crust coated with S2 also revealed a homogenous background of starch, but
400 the microcapsules appeared more concentrated, leading to denser zones, as was observed
401 in the coating microstructure (Figure 4e). The sample treated with S3 showed higher
402 density of microcapsules over the surface (Figure 4g) comparing with the other samples,
403 which might be attributed to the high concentration of microcapsules used in this
404 treatment. Less difference was observed in the cross section micrographs (Figure 4 b, d, f,
405 h). A compact cross section with some small void spaces were observed in all the
406 micrographs, although less void spaces were observed in the samples with double starch
407 layers (Figure 4 f, h). In the crust cross sections the microcapsules were observed onto the
408 surface, and in sample with S3 the microcapsules were more embedded in the gelatinized
409 matrix. Thus it seems that the microcapsules were better covered when they were located
410 between two starch layers, which agree with the survival results described in section 3.2.
411
412 **4. Conclusion**
413 Overall results show that *Lactobacillus acidophilus* included in microcapsules can be
414 incorporated to bread surface through edible coatings, leading to bread with similar
415 characteristics to common bread, but with additional healthy benefits. Edible coatings

have been used as a vehicle for microorganism and the physical properties of the resulting bread confirmed the potential use of this procedure for obtaining healthier baked goods. The survival of microencapsulated *Lactobacillus acidophilus* demonstrated the ability of starch solution to protect the microcapsules during baking and storage time, likely due to the adhesion of the microcapsules to the starch macromolecules. This study also shows that the functionality of edible coatings depends on their composition (suspension constituents) and the coating procedure (monolayer, successive layer or multi coating) onto the product. Considering the microorganism survival, the physico-chemical properties of the bread crust and the economy of the process, the treatments S1 and S2 would be the best alternative for carrying the microcapsules. Currently, studies are undertaken to confirm the probiotic effect of these breads by carrying out *in vitro* and *in vivo* studies.

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References

Altamirano-Fortoul, R., and Rosell, C. M. (2011). Physico-chemical changes in breads from bake off technologies during storage. *LWT - Food Science and Technology, 44*, 631-636.

441 ASTM. (2001). Standard test methods for tensile properties of thin plastic sheeting.
442 Standards Designation: D882-00. In Annual Book of ASTM, Philadelphia, PA.
443 Bertuzzi, M.A., Castro Vidaurre, E.F., Armada, M., and Gottifredi, J.C. (2007). Water
444 vapor permeability of edible starch based films. *Journal of Food Engineering, 80*,
445 972-978.
446 Capela, P., Hay, T.K. C., and Shah N.P. (2006). Effect of cryoprotectants, prebiotics and
447 microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried
448 yoghurt. *Food Research International, 39*, 203–211.
449 Cocato, M.L., Ines Re, M., Alves Trindade Neto, M., Pontes Chiebao, H., and Colli, C.
450 (2007). In vitro and in vivo evaluation of iron bioavailability from microencapsulated
451 ferrous sulfate. *Revista de Nutricao, 20*, 239-247.
452 Craig, S.A., Maningat, C.C., Seib, P.A., and Hoseney, R.C. (1989). Starch paste clarity.
453 *Cereal Chemistry, 66* (3), 173-182.
454 Cuq, B., Aymard, C., Cuq, J.L., and Guilbert, S. (1995). Edible packaging films base on
455 fish myofibrillar proteins: formulation and functional properties. *Journal of Food*
456 *Science, 60(6)*, 1369-1374.
457 De Jonge, J., Amorij, J., Hinrichs, W., Wilschut, J., Huckriede, and Frijlink, H. (2007).
458 Inulin sugar glasses preserve the structural integrity and biological activity of
459 influenza virosomes during freeze-drying and storage. *European Journal of*
460 *Pharmacology Science, 32*, 33-44.
461 Goedeken, D.I. (1993). Permeability measurements of porous food materials. *Journal of*
462 *Food Science, 58*, 1329-1331.
463 Gomes, A.M.P., and Malcata, F.X. (1999). Bifidobacterium spp. and Lactobacillus
464 acidophilus: biological, biochemical, technological and therapeutical properties
465 relevant for use as probiotics. *Trends in Food Science & Technology* 10, 139-157.

466 Guarner, F., and Schaafsma, G.J. (1998). Short communication probiotics. *International*
467 *Journal of Food Microbiology, 39,* 237–238.
468 ICC. (1994). Standard methods of the International Association for Cereal Science and
469 Technology, Vienna, Austria.
470 Jackman, R.L., and Stanley, D.W. (1992). Area and perimeter dependent properties and
471 failure of mature green and red ripe tomato pericarp tissue. *Journal of Texture*
472 *Studies, 23,* 461-474.
473 Lavermicocca, P., Valerio, F., Lonigro, S.L., De Angelis M., Morelli, L., Callegari, M.L.,
474 Rizzello, C.G., and Visconti, A. (2005). Study of adhesion and survival of
475 lactobacilli and bifidobacteria on table olives with the aim of formulating a new
476 probiotic food. *Applied and Environmental Microbiology, 71,* 4233-4240.
477 Lian, W.C., Hsiao, H.C., and Chou, C.C. (2002). Viability of microencapsulated
478 bifidobacteria in simulated gastric juice and bile solution. *International Journal of*
479 *Food Microbiology, 86,* 293-301.
480 Martínez, T. R., and Morales, D. U. R. (2007). Study of dehydratation of Maguey (Agave
481 atrovirens Karw) in order to obtain a dried product with high quantity of
482 Fructooligosaccharides. *Bebidas, Alfa Editores Técnicos, 2,* 11-19.
483 Müller, C.M.O, Laurino, B.J., and Yamashita, F. (2009). Effect of cellulose fibers addition
484 on the mechanical properties and water vapor barrier of starch-based films. *Food*
485 *Hydrocolloids, 23,* 1328–1333.
486 Primo-Martin, C., Beukelaer, H., Hamer, R.J., and Van Vliet, T. (2008). Fracture
487 behaviour of bread crust: effect of ingredient modification. *Journal of Cereal*
488 *Science, 48,* 604-612.
489 Quezada-Gallo, J.A., Bon Rosas, F., Ramírez Gómez, M., Díaz Amaro, M.R., Noah, N.,
490 and Datzenko, A. (2004). Performances of edible coatings combined with bread

491 additives to preserve fried donuts and mexican white bread “bolillo”. *Proceedings of*
492 *the AACC Conference* (San Diego, CA, USA).
493 Redgwell, R., and Fischer, M. (2005). Dietary fibre as a versatile food component: an
494 industrial perspective. *Molecular Nutrition Food Research, 49,* 421-435.
495 Rodríguez-Huezo, M., Duran-Lugo, R., Prado-Barragan, L., Cruz Sosa, F., Lobato-
496 Calleros, C. Alvarez-Ramirez, J., and Vernon-Carter, E. (2007). Pre-selection of
497 protective colloids for enhanced viability of Bifidobacterium bifidum following
498 spray-drying and storage, and evaluation of aguamiel as thermoprotective prebiotic.
499 *Food Research International, 40,* 1299-1306.
500 Rosell, C.M. (2007). Vitamin and mineral fortification of bread. In: Technology of
501 Functional Cereal Products. Ed B. Hamaker. Woodhead Publishing Ltd, Cambridge,
502 UK.
503 Rosell, C.M. (2009). Trends in breadmaking: Low and subzero temperatures. In:
504 Innovation in Food Engineering: New Techniques and Products. Ed M.L. Passos,
505 C.L. Ribeiro. Taylor and Francis, CRC Press. Pp 59-79.
506 Shafiee, S., Motlagh, M, A., Didar, A.R., and Minaee, S. (2008). Investigation the effect
507 of skin on mechanical behavior of apple. *Journal of Food Technology, 6*, 86-91.
508 Stokes, D.J., and Donald, A.M. (2000). In situ mechanical testing of dry and hydrated
509 breadcrumb in the environmental scanning electron microscope (ESEM). *Journal of*
510 *Materials Science, 35*, 311-321.
511 Tharanathan, R.N. (2003). Biodegradable films and composite coatings: past, present and
512 future. *Trends in Food Science and Technology*, 14, 71–78.
513 Veiga-Santos, P., Oliveira, L. M.; Cereda, M. P., Alves, A. J., and Scamparini, A. R. P.
514 (2005). Mechanical properties, hydrophilicity and water activity of starch-gum films:

Effect of additives and deacetylated xanthan gum. *Food Hydrocolloids, 19*, 341 - 349.

Villa-García, M., Pedroza-Islas, R., Moreno-Terrazas R., De la Rosa-Miranda M., and Martínez-Ferez, A. (2010). Polidextrosa, inulina y aguamiel de maguey y su influencia en la sobrevivencia de Lactobacillus acidophilus microencapsulado en una mezcla de biopolímeros. BiopMat-Biopolímeros: fuentes, transformación, producción y aplicaciones innovadoras. Fundación para la Educación Superior Internacional, A. C. Electronic book access: www.fesi.org.mx.

FIGURE CAPTIONS

Figure 1. Digital images of probiotic coatings. The composition of a) S1, b) S2 and c) S3 is detailed in Materials and methods section.

Figure 2. Scanning electron micrograph of starch-based coating film. Legends: (a): Microcapsules, (b): Surface of a starch coating film containing microcapsules, (c): Cut side of S1 coating film, (d): Cut side of S2 film, (e): Cut side of S3 film.

Figure 3. Physico-chemical properties of the probiotic bread crusts during a short storage at 25ºC. (**a)**: moisture content vs. time, (**b)**: water activity vs. time, (**c)**: failure force vs. time. S1, S2, S3 are referred to the different probiotic coatings applied to the bread surface.

Figure 4. Scanning electron micrographs of bread crust surface (**a, c, e, g**) and cross section (**b, d, f, h**). Images correspond to the following probiotic coatings treatments: (**a, b)**: Crust without probiotic coating, (**c, d)**: crust with S1 treatment, (**e, f)**: crust with S2 treatment, **(g, h)**: crust with S3 treatment. Scale bars of 30µm.

542 **Table 1.** Probiotic coatings concentrations applied onto the bread surface

Sample	Probiotic Coatings	Dosage
S1	1) Starch-microcapsules solution	5% starch containing 1% microcapsules
S2	1) Starch-microcapsules solution	5% starch containing 1% microcapsules
	2) Starch solution without microcapsules	5% starch
S3	1) Starch solution without microcapsules	5% starch
	2) Microcapsules sprayed	2% microcapsules
	3) Starch solution without microcapsules	5% starch

543
544

545 **Table 2**. Survival of *Lactobacillus acidophilus* after 24 h of short-term storage.
546

Samples	Concentration of *Lactobacillus acidophilus*[a] in each bread	Fresh bread (CFU/bread)	24h stored bread (CFU/bread)
Control	0.00E+00	0.00E+00	0.00E+00
S1	4.83E+07	2.40E+07	1.70E+06
S2	4.83E+07	3.05E+07	1.15E+06
S3	9.66E+07	2.75E+07	1.22E+06

547

548 [a]The initial concentration of *Lactobacillus acidophilus* in the microcapsule was
549 4.83E+08 UFC/g.

550 **Table 3**. Characteristics of fresh bread treated with probiotic coatings.

Sample	Control	S1	S2	S3
Specific volume ml/g	2.9 b	2.9 b	2.8 a	2.9 b
*L**	61.7 c	61.3 c	57.9 b	56.4 a
*a**	11.9 b	12.1 b	10.4 a	10.3 a
*b**	37.0 b	37.6 b	25.8 a	24.5 a
Crust A_w	0.43 a	0.54 b	0.54 b	0.56 c
Crust moisture content (%)	7.9 b	9.4 c	6.6 a	9.9 d
Failure force (N)	13.1 d	11.4 c	9.6 a	10.6 b
Failure deformation (mm)	4.3 b	3.4 a	5.1 c	4.9 c
Failure firmness (N/mm)	2.6 c	3.4 d	1.9 a	2.2 b
Sensory analysis				
Crust appearance	4.1 a	3.1 a	3.7 a	3.1 a
Odour	3.7 a	3.7 a	3.1 a	3.9 a
Crust colour	4.0 a	3.3 a	3.6 a	2.7 a
Crispiness	4.3 a	3.9 a	3.1 a	3.9 a
Crumb hardness	4.1 a	3.4 a	4.0 a	3.9 a

551

552 Means sharing the same letter within a row were not significantly different ($p<0.05$).
553

Figure 1.

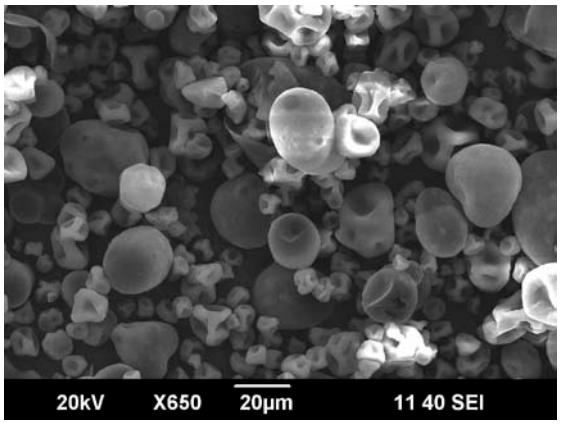
a

b

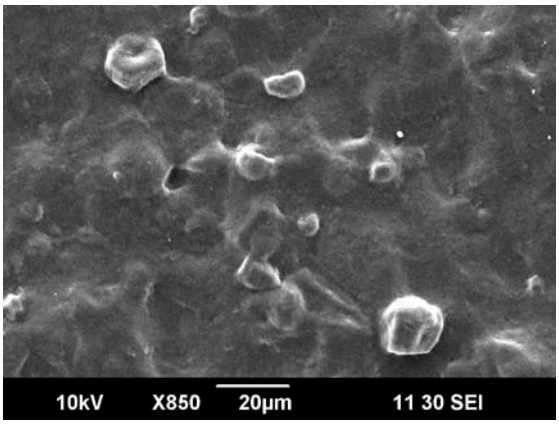
c

Figure 2.

a



b



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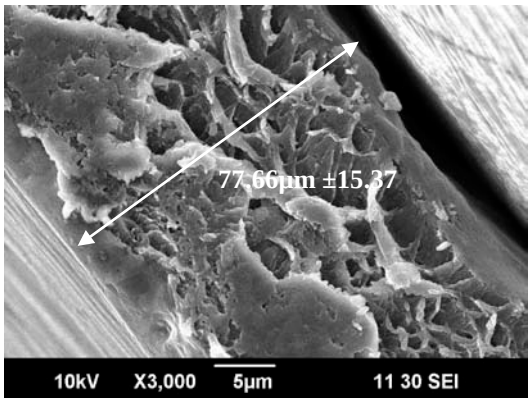

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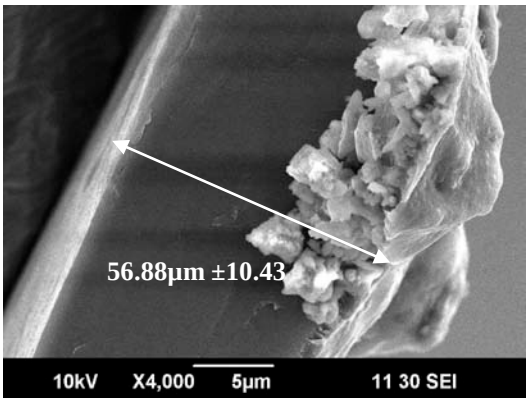

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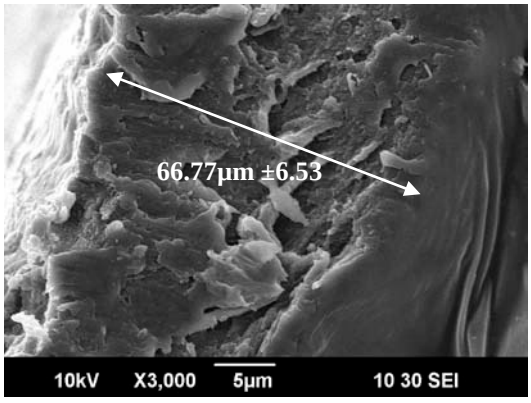
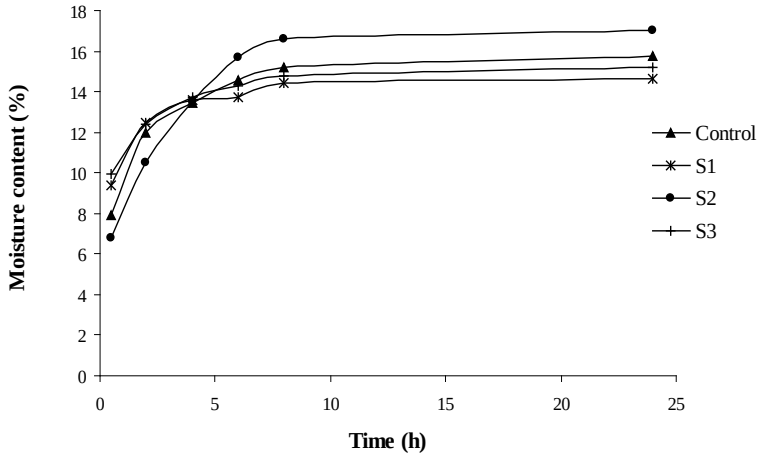
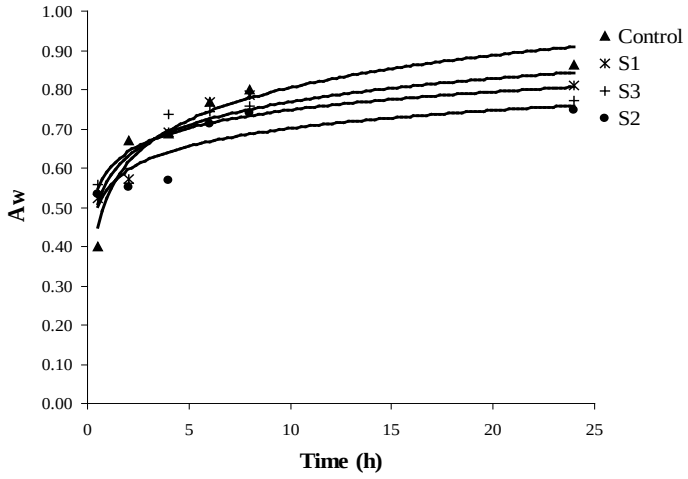


Figure 3.

a.


Moisture content (%)
Time (h)
Control
S1
S2
S3

b.


Aw
Time (h)
Control
S1
S3
S2

c.

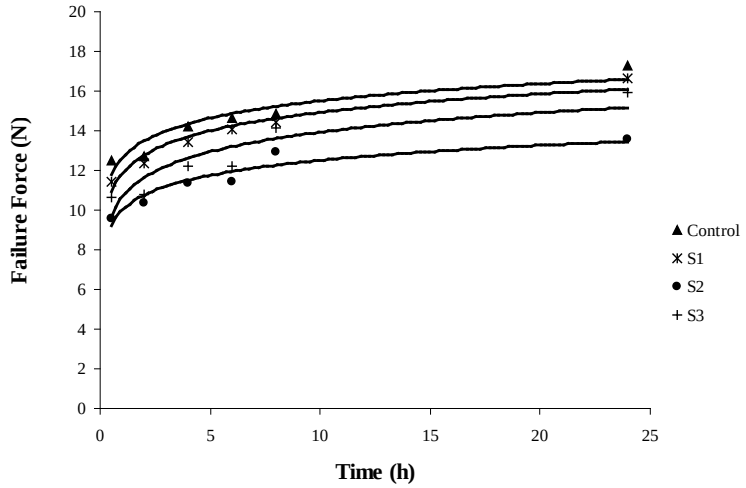
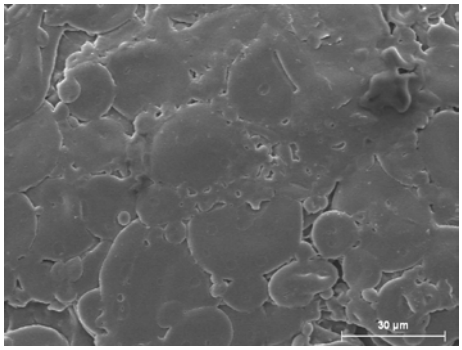

Failure Force (N)
Time (h)
Control
S1
S2
S3

Figure 4

a



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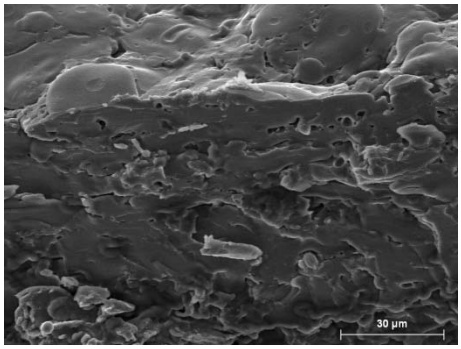

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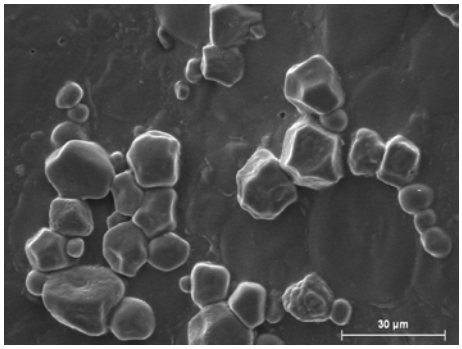

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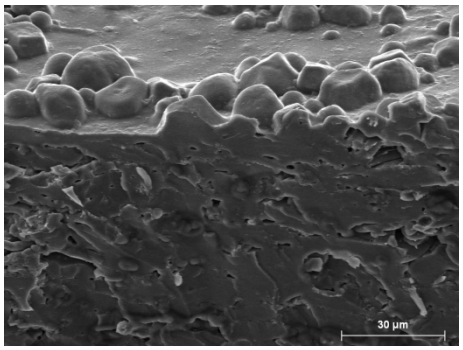

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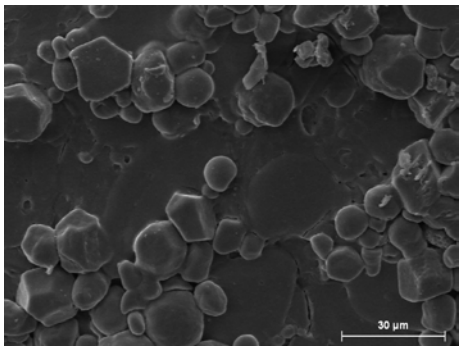

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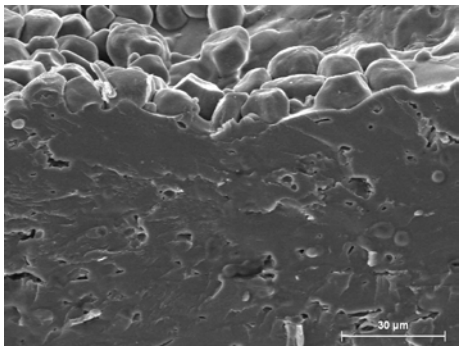
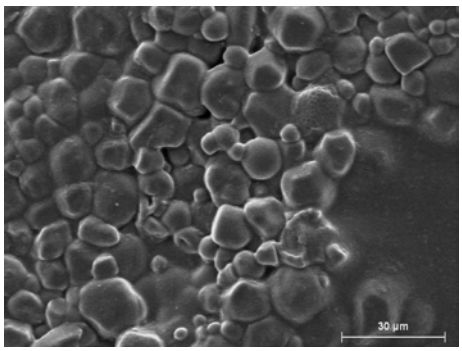


g)



h)