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Application of coating on dog biscuits for extended survival of probiotic bacteria

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ABSTRACT

Functional biscuits for adult domestic dogs containing probiotic bacteria (*Lactobacillus plantarum*) and prebiotics (inulin) were developed and characterized. In order to assure a higher bacterial survival, the application of a protective coating was necessary. Two biscuit formulations were prepared, one with wheat flour and the other with soy and whole wheat flour. After baking them at 140 °C for 45 min, hard and crispy biscuits appropriate for adult dogs were obtained. Biscuits were treated either with calcium alginate or starch–glycerol coating. The preferred coating formulation was the starch–glycerol because of a higher survival rate of *L. plantarum* observed after passing through a simulated gastrointestinal system (HCl pH 1–2). In the case of wheat flour biscuits with coating, the bacteria survival rate after passing through the *in vitro* system, showed higher survival rate, regardless the presence of inulin while in soy and whole wheat flour biscuits, the presence of coating did not improve bacteria survival. The latter formulation showed a protective effect thus obtaining higher bacteria survival without coating. It is worth mentioning that after one month storage, biscuit formulations with coating maintained viable counts of *L. Plantarum* higher than 10^8 CFU/mL. The presence of inulin at 20 g/L did not show a significant effect on the survival rate of bacteria after one month storage period.

1. Introduction

Nowadays, pets are in millions of homes worldwide and as a consequence an increasingly sophisticated pet food industry developed focusing mainly on pet' nutrition needs. Domestic animals have only limited freedom to choose their own diets and to a certain degree are dependent on the judgement, prejudice or will of their owners. Other criteria influencing the food choice for dogs and cats are more related to those for human food. Pets share many of the foods that their owners enjoy, and the owner assumes that there is much in common between his/her own likes and dislikes and those of his/her dog (Rofe and Anderson, 1970). Moreover, owners prefer buying dried pet food due to its shelf-life, nutrient content and because it is cheaper (Robertson, 1996).

Abbreviations: a_w, water activity; CFU, colony forming units; FOS, fructooligosaccharides; GOS, glucooligosaccharides; GRAS, generally recognized as safe; MRS, de Man, Rogosa and Sharpe; W, wheat flour biscuits; WWS, soy and whole wheat flour biscuits.

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According to the definition approved by the National Yogurt Association, NYA, and by the International Life Science Institute, ILSI (2002), probiotics are live organisms, which when consumed in adequate amounts confer a healthy benefit on the host apart from basic nutrition (Garrote et al., 2001). The status of probiotics, as a food component, is currently not established on an international basis, because government regulations differ among countries (FAO/WHO, 2002). The FDA (Food and Drug Administration) recognized them as GRAS ingredients that have no established maximum amounts in food. Probiotics have been incorporated into a wide range of foods including yogurt, cheese, ice cream, and non-dairy products such as chocolate, fruit, biscuits, meat, *etc.* (Burgain et al., 2011). Gismondo et al. (1999) studied the main effects on the host's health of the living lactic acid bacteria when present in the intestinal tract. This fact is attributed to the competition for receptor sites of adherence to the intestinal epithelium, to the production of antibacterial substances (organic acids and antibiotics) and/or stimulation of the immune system (Perdigón et al., 1990). Perdigón et al. (1993) reported that there is sufficient evidence to confirm that lactobacilli strains or dairy products fermented with these organisms inhibit tumor cell lines in mice. Garrote et al. (2001) have studied the probiotic effects of *Lactobacillus plantarum*, which can colonize the gastrointestinal tract of humans and mammals. Besides, *L. plantarum* is an important species involved in the fermentation of vegetable products (Ashenafi and Busse, 1991) and is well known due to its ability to produce antimicrobial substances, such as plantaricins, which are active against certain pathogenic organisms (Muck, 1996).

Prebiotic foods are defined as non-digestible food ingredients that benefit the host by stimulating the growth and activity of one or of a limited number of bacteria in the colon, improving in this way the host's health (Gibson and Roberfroid, 1995). Examples of these ingredients are: fructooligosaccharides (FOS) and glucooligosaccharides (GOS). Spiegel et al. (1994), Strickling et al. (2000) and Yu Wang et al. (2010) reported that FOS consumption leads to a better absorption of several minerals, such as calcium and magnesium, essential components in bones and teeth. Among FOS, inulin is widely used in the food industry as a soluble, odourless and hypoallergenic fibre. Combination of these specific chemical substances with the administration of probiotics produces a synergistic effect on a wide variety of metabolic processes (Tomomatsu, 1994). Related to this, Takemura et al. (2010) found that inulin stimulated the growth of *L. Plantarum* in the gastrointestinal tract of a mouse.

When adding probiotics to foods, the survival of bacteria during processing and while exposed to gastric conditions should be considered. Several techniques involving encapsulation and immobilization of bacteria have been used so that a larger number of viable bacteria can reach the intestine (Gilson and Thomas, 1995; Sultana et al., 2000). Starch, alginate, carrageenan and chitosan are included among the hydrocolloids used to encapsulate or to obtain films and coatings (Iyer and Kailasapathy, 2005; George and Abraham, 2006; Chen and Subirade, 2006; Ding and Shah, 2007; Burgain et al., 2011). The existing knowledge of prebiotic and probiotic use in food could help to develop beneficial applications for other mammals, like domestic animals.

In addition to food, pets are usually awarded with biscuits. The incorporation of prebiotics and probiotics could turn these biscuits into a functional food. Thus, the aim of the present work was to obtain a biscuit for healthy adult dogs, containing *Lactobacillus plantarum* and inulin, protected with a coating to increase bacteria survival after gastro-intestinal digestion.

2. Materials and methods

2.1. Biscuit formulation

Two dough formulations were prepared: "W" contained 495 g/kg wheat flour (Favorita, Molinos Río de La Plata S.A., Argentine), 10 g/kg NaCl (Dos Anclas, Argentine), 74 g/kg sucrose (Ledesma, Argentine), 8 g/kg sodium bicarbonate (Anedra, Argentine), 18 g/kg sodium phosphate monobasic (Anedra, Argentine), 99 g/kg sunflower oil (Cocinero, Molinos Río de La Plata S.A., Argentine) and 296 g/kg distilled water; and formulation "WWS" contained 247.5 g/kg whole wheat flour (Pureza, Molinos Cañuelas, Argentine), 247.5 g/kg soy flour (Yin-Yang, Argentine), 10 g/kg NaCl (Dos Anclas, Argentine), 74 g/kg sucrose (Ledesma, Argentine), 8 g/kg sodium bicarbonate (Anedra, Argentine), 18 g/kg sodium phosphate monobasic (Anedra, Argentine), 99 g/kg sunflower oil (Cocinero, Molinos Río de La Plata, SA, Argentine) and 296 g/kg distilled water.

All dry components were weighed separately and homogenized; then the oil and the water were incorporated. All ingredients were manually mixed to obtain soft dough. The dough was allowed to stand for 15 to 20 min, rolled out on a levelling plate of 0.5 cm height and cut using a circular cutter (3 cm in diameter). Samples were then placed on baking pans. Finally, a glass rod was used to make a small cavity in the centre of each biscuit so as to be able to add the suspension of probiotics after baking.

Two baking conditions were used: 140 °C–45 min and 180 °C–30 min. The convection oven (ARISTON FM87-FC, Italy) was pre-heated until reaching the baking temperature before each test. The thermal histories were recorded with Cu-constantan thermocouples placed in the centre of the control samples.

2.2. Addition of potential probiotics and prebiotics on the biscuits

The strain *L. plantarum* CIDCA 83114, isolated from granules of kefir CIDCA AGK1, from the Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), was used. This strain has proved to inhibit *Salmonella* and *Escherichia coli in vitro*, also it has been able to adhere to Caco-2 cells (Golowczyc et al., 2008) and antagonize *E. coli* O157:H7 on

Hep-2 cells (Hugo et al., 2008); also when combined with *Streptococcus thermophilus* it antagonized cytopathogenesis by enterohaemorragic *E. coli* Shiga toxin in cultures of Vero cells (Kakisu et al., 2013).

L. plantarum culture, once reactivated, was inoculated with an automatic pipette in MRS (De Man et al., 1960) broth (1 mL/100 mL MRS) at 30 °C for 15 h. These conditions allowed a concentration of approximately 10^9 CFU (colony forming units) per mL (Shi et al., 2013).

Fractions of 1 mL of bacterial suspension were centrifuged at 20400 g for 5 min at 25 °C and supernatants were discarded. Then, the pellets were re-suspended in 0.1 mL of MRS broth and agitated in a vortex. Under sterile conditions, the bacterial suspension was placed on a cooked biscuit using an automatic pipette. The whole volume was deposited in the previously formed depression on the biscuit surface.

A suspension with bacteria and inulin was added to a portion of the biscuits. For this purpose 20 g/L inulin (Frutafit®-inulin, Netherland) was solubilized in MRS broth, homogenized with a vortex for approximately 3 min and then an aliquot of 0.1 mL was used to re-suspend pellets of *L. plantarum*. Then, this suspension of bacteria with inulin was placed under sterile conditions on the cooled and dried surface of a set of cooked biscuits.

2.3. Coating preparation

To obtain the highest bacteria survival on the biscuits, two coatings were tested. On one hand, calcium alginate coating on the biscuits was obtained by spreading firstly with a brush 20 g/L sodium alginate (Sigma Aldrich, USA) and then spraying a solution of 0.05 M $CaCl_2$ to form a gel. The coated biscuits were dried in the oven at 30 °C for 40 min.

On the other hand, a gelatinized suspension of corn starch (Unilever, Argentine) was spread on the biscuits. The suspension (30 g/L corn starch) was gelatinized in a thermostatic bath (Haake, Germany) at 90 °C for 30 min. When the suspension reached room temperature, 0.9 g of glycerol per L of suspension was added. Then, the coated biscuits were dried in the oven at 30 °C for 40 min.

2.4. Viability of the bacteria on biscuits after passage through simulated gastrointestinal fluids

Survival of the bacteria on the biscuits was analyzed in simulated gastric and intestinal fluid systems. Ten grams of biscuits and 40 mL of triptone at 1 g/L were placed in Stomacher plastic bags. The mixture was homogenized in the Stomacher (Seward Laboratory Blender, Stomacher 400, UK) for 90 s. Then, 5 mL was transferred to an Erlenmeyer and 5 mL of the simulated gastric fluid (HCl, pH 1.8) was added; the mixture was incubated at 37 °C for 3 h in an orbital shaker (Orbit Environ Shaker, Lab Instruments, USA) at 180 rpm. Then, an aliquot of 5 mL was transferred to the simulated intestinal fluid (phosphate buffer, pH 7.4) and agitated at 180 rpm in the orbital shaker for 2 h at 37 °C.

The concentration of viable microorganisms was determined by the dish count method. Dissolution series were conducted with 1 g/L triptone. Petri plates with MRS agar were inoculated with 0.1 mL of each dilution and then, incubated at 30 °C in aerobic conditions. Colony counts were carried out after 24 h. Results were expressed as colony forming units by millilitre or (CFU/mL). Counting of viable bacteria was done in duplicate at 30 °C after 24 h incubation.

In each assay, viable microorganisms of control biscuits without coating were determined following the same procedure above described.

2.5. Bacterial survival during storage

Viability of bacteria with and without inulin was studied on biscuits stored at 20 °C for 28 days. Control biscuits without coating were also analysed.

Besides, the bacterial survival after *in vitro* digestion was also determined on coated biscuits containing *L. plantarum* and inulin after 28 days storage.

All the assays were done in triplicate.

2.6. Characterization of the biscuits

Starch gelatinization was monitored by optical microscopy with polarized light so as to be able to choose the proper cooking conditions, taking into account that a complete gelatinization is needed for this type of product. The samples were previously ground in a mortar, and then placed on a microscope slide for observation under a Leica DMLB microscope (Heerbrugg, Germany), equipped with a Leica digital camera DC 100.

Surface colour was measured with a colorimeter (Minolta CR-400, Minolta Corp., Ramsey, NJ, USA). Luminosity L^* and chromaticity parameters a^* and b^* were determined. Mean values were calculated from at least 10 samples and each test was repeated at least three times.

Texture of biscuits was analysed using a texture analyser TA.XT2i (Stable Micro Systems, UK). In addition, a fracture test was carried out under the following conditions: pre and post-assay velocity: 1 mm/s, testing velocity: 0.5 mm/s, maximum compression percentage was 60%. A compression probe of 1×0.02 cm was used.

Mean values were calculated from at least 10 samples. The maximum fracture force and the slope at the maximum were determined from the force-deformation curve.

The integrity of coating applied on the biscuits was assessed using a stereomicroscope (Leica MZ 10 F, Germany). Photographs were taken with an attached camera (Leica DFC 490, Germany) and an iodine–iodure solution was used to highlight the presence of the starch coating.

Water activity (a_w) was determined using Aqua Lab (Series 3 TE, USA) equipment. Calibration was conducted with saline solutions of a known a_w.

Moisture content was determined using 5 g of biscuits. They were placed in the oven at 105 °C until a constant weight was achieved. Percentage moisture on a dry basis was calculated according to the following equation:

$$H(\%) = \frac{(W_i - W_f) \times 100}{W_f} \quad (1)$$

$H(\%)$ is the moisture percentage, W_i is the initial weight of the sample, W_f is the final weight of the sample.

2.7. Statistical analysis

Data analysis was performed with the software SYSTAT INC. version 12 (Evenston, IL, USA). Monofactorial and bifactorial designs were used considering interaction between factors in the second case. Analysis of variance (ANOVA) and mean comparisons (LSD and Tukey) were carried out. Unless indicated, a level of 95% of confidence ($\alpha = 0.05$) was used.

3. Results

Starch granules increased their size 10 times after baking. Birefringence loss of the granules was observed after the baking process indicating a complete gelatinization under both assayed conditions (data not shown). Therefore, to select the more proper cooking condition, texture and colour of the baked biscuits were taken into account. Macroscopic observation indicated that both conditions showed acceptable colour and texture for the desired product (Fig. 1). All the luminosity values were around 60 ($P > 0.05$). Chromaticity was compared using a^*/b^* parameters, and values were around 0.2 ($P > 0.05$).

Fig. 1 shows typical force–deformation profiles for both biscuit formulations under both baking conditions. ANOVA tests conducted for the maximum force and for the slope showed that both the type of flour and the baking condition were significant factors ($P < 0.05$) (Fig. 2a and b). When using Fisher test (LSD) to compare mean values, the slopes showed significant difference ($P < 0.05$) between baking conditions and flour (Fig. 2b). However, under both baking conditions WWS samples were not significantly different for maximum resistance (Fig. 2a).

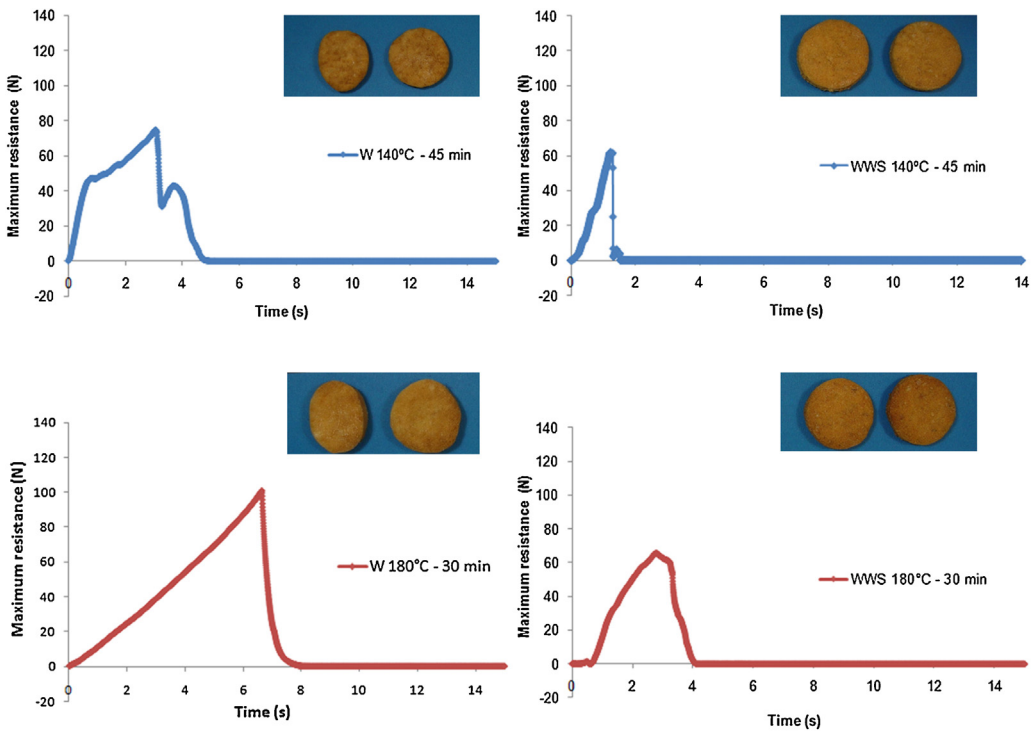

Fig. 1. Texture profile and photographs of recently baked biscuits under both baking conditions.

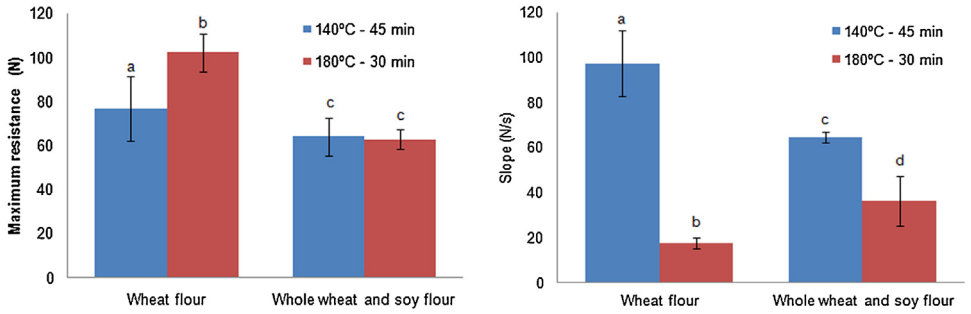

Fig. 2. Effect of the baking conditions on the texture of W and WWS biscuits. W—wheat flour, WWS—soy flour and whole wheat flour. (a) Maximum resistance (N) (b) Slope (N/s) of the resistance *vs.* time. *Different superscripts indicate that the average values differ significantly ($P < 0.05$).

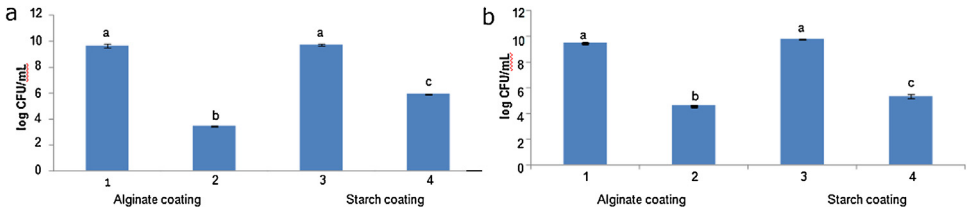

Fig. 3. Effect of the type of coating applied to biscuits on the count of colonies of *L. plantarum* before ((1) and (3)) and after ((2) and (4)) *in vitro* digestion. (a) Biscuits made with wheat flour. (b) Biscuits made with soy flour and whole wheat flour. *Different superscripts indicate that the average values differ significantly ($P < 0.05$).

3.1. Coating selection

The colonies of *L. plantarum* showed typical appearance: convex, white and bright. The colonies exhibited the same appearance before and after *in vitro* digestion. *L. plantarum* in MRS broth reached approximately 10^9 CFU/mL after 15 h incubation at 30 °C. A specific viable cell concentration was deposited onto each biscuit to accurately calculate the effect of simulating gastrointestinal fluids. Besides, the calcium alginate and starch coatings were tested so as to be able choose the one which would keep the highest bacteria viability after the *in vitro* digestion. After 40 min at 30 °C drying, both film-forming formulations became almost invisible dried coatings protecting the bacteria.

The effect of coating formulation on *L. plantarum* survival was evaluated before and after simulated digestion (Fig. 3a and b). For W biscuits with alginate coating the counts decreased from 9.5 log CFU to 3.5 log CFU, while with starch coating a better performance was obtained decreasing from 9.5 log CFU to 5.5 log CFU (Fig. 3a). A similar trend was obtained with WWS biscuits (Fig. 3b). From these results, starch-based coating was chosen for the rest of the tests.

The effect of the presence of coating and inulin, added to bacteria suspension, was evaluated on bacterial survival after *in vitro* digestion. Since the logarithm of the initial count of all the tests was approximately 9.7, the reduction in the log of counts (Δ orders of magnitude, Eq. (2)) caused by each treatment was represented in Fig. 4a and b. A higher value in the Δ indicates a higher loss of bacteria due to the simulated gastrointestinal fluids and thus, a lower protection of the system.

$$\Delta \text{ orders of magnitude (UFC/mL)} = \log \text{(UFC/mL) initial} - \log \text{(UFC/mL) after in vitro digestion} \quad (2)$$

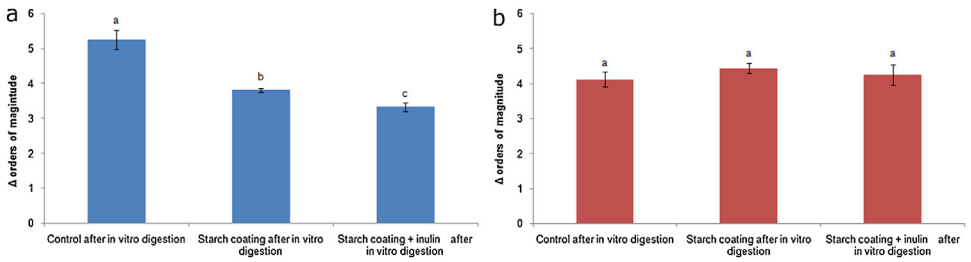

Fig. 4. Effect of *in vitro* digestion on bacterial survival. (a) Biscuits made with wheat flour. (b) Biscuits made with whole wheat flour and soy flour. *Different superscripts indicate that the average values differ significantly ($P < 0.05$).

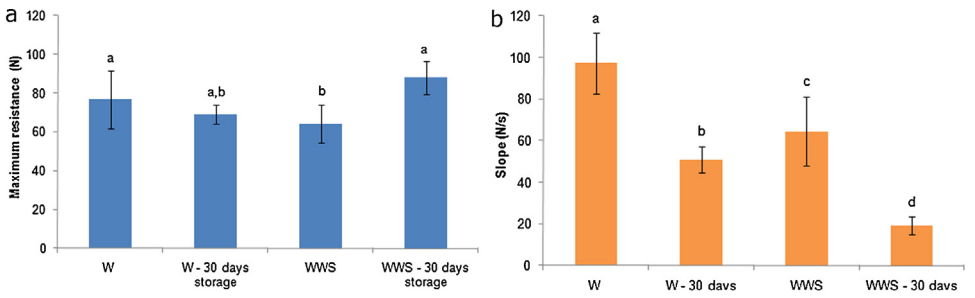

Fig. 5. Effect of storage on the texture parameters of biscuits made with wheat flour and biscuits made with whole wheat flour and soy flour). (a) Maximum resistance (N), (b) slope (N/s) of the resistance *vs.* time. * Different superscripts indicate that the average values differ significantly ($P < 0.05$).

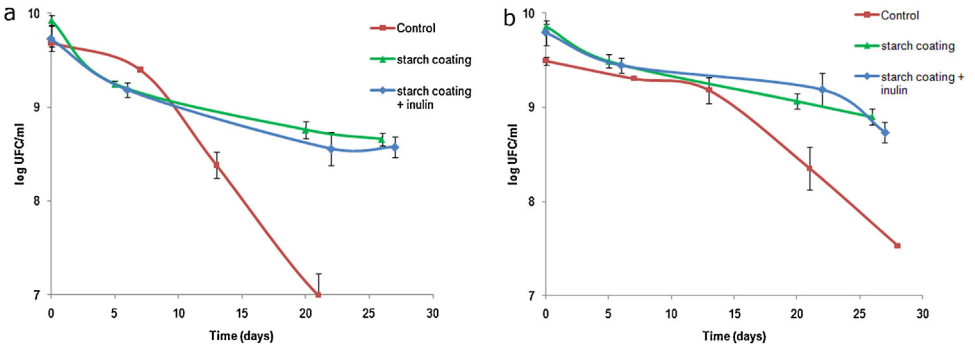

Fig. 6. Effect of storage on the counts of *L. plantarum* in control biscuits and with starch-based coating. (a) Biscuits with wheat flour. (b) Biscuits with whole wheat flour and soy flour.

In the case of W biscuits (Fig. 4a and b), the coating presence was always favourable, maintaining the counts ranging from 1 to 2 orders of magnitude higher than that of the control. Using Tukey test, the best result was obtained with biscuits containing inulin and starch coating ($P < 0.05$). In the case of WWS biscuits, either with or without inulin, coating addition was not a significant factor ($P > 0.05$) for bacterial survival under *in vitro* digestion.

3.2. Storage

To analyze the effect of coating on the biscuit texture during storage, biscuits were prepared by adding the same amount of MRS broth (without bacteria) as used for biscuit digestion, before adding the coating. Fig. 5a and b shows the maximum resistance and the slope of both types of biscuits before and after 30-day storage. The maximum resistance, associated with the biscuit hardness, showed significant differences regarding storage time or the type of formulation. Besides, the type of flour and storage proved to be a significant factor ($P < 0.05$) for the slope, which indicated that storage reduce product crispiness.

After a month of storage at 20 °C, water activity was approximately 0.60 for both types of biscuits, values similar to those determined before storage. At the same time, water content was 112.6 g/kg on dry basis for biscuits made with wheat flour and 113.0 g/kg for those made with whole wheat flour and soy flour.

Regarding *L. plantarum* survival during storage, Fig. 6 shows the beneficial effect of coating presence. For W coated biscuits with and without inulin (Fig. 6a), the initial count was around 9.8 log CFU and after 20 days of storage was 7.2 log CFU for the control, and approximately 8.7 log CFU for coated biscuits. For WWS coated biscuits with and without inulin (Fig. 6b), the difference in bacterial survival between the control and the coated biscuits was not so marked. The addition of inulin was not a significant factor ($P > 0.05$).

To study the viability of *L. plantarum* bacteria on stored biscuits, an additional digestion assay was performed on biscuits with inulin and starch coating, after 28 day storage. Bacteria survival showed no significant differences in the relative reduction of the orders of magnitude between just prepared and stored biscuits.

4. Discussion

The type of flour used showed a different cooking behaviour during the baking process that could be associated with its physicochemical properties. Conforti et al. (2012) also studied the effect of the composition on the cooking behaviour of biscuits with wheat or corn starch, and found that the temperature of the centre of the biscuit was lower than the oven

temperature, showing a temperature gradient between the surface and the centre of the sample. Those authors also found that the presence of corn starch increased the maximum temperature in the centre of the baking biscuit and decreased the baking time. In the present work, under the same conditions of time and temperature the dough made with wheat flour reached a higher temperature in the thermal centre than that recorded for the WWS dough. For a baking temperature of 140 °C, the thermal centre reached 123.1 °C for the W dough, while for the WWS dough it was 114.6 °C.

In spite of the mentioned differences on cooking behaviour, both baking conditions showed a total starch gelatinization for both formulations: when dealing with animal food, to avoid digestive problems, an important parameter should be assuring complete gelatinization of starch.

In order to choose the most adequate baking conditions, only texture analysis was taken into account since colour did not show significant differences. Baking at 140 °C–45 min gave harder and crispier products regardless the type of dough. Case et al. (2001) reported that a hard texture favours teeth hygiene, which is associated with animal health and wellbeing.

Several researchers who studied biscuit crispiness and other snack products with instrumental methods found that among the parameters obtained from the resistance–deformation curve, the best indicator of crispiness was the slope (Katz and Labuza, 1981). Sarantópoulos et al. (2002) associated the texture profiles of different materials with the mechanic properties. This classification and the form of the maximum resistance profiles and initial slope reveal that at 140 °C–45 min a more rigid and crispy structure was obtained, which was quite different to the one obtained at 180 °C–30 min, which was more plastic and more deformable. In the case of *W* biscuits, the difference between the slope values was higher than with WWS biscuits, revealing the influence of the type of formulation on the baking conditions.

4.1. Coating efficiency

Calcium alginate successfully formed a coating on the biscuits after drying. Similarly, Sriamornsak and Kennedy (2006) have developed a method to produce wet gel films of calcium alginate and obtained a dried coating on a surface, and have analyzed the coating rehydration in either water or simulated gastric fluid.

However, starch-based formulation was the preferred coating because it showed the highest number of viable colonies after exposure to an extreme simulated gastrointestinal environment. Similar starch-based coatings developed in our laboratory helped extending the shelf life of fresh products. (García et al., 2001, 2009).

In order for the bacteria to exert a probiotic effect, the International Dairy Federation has recommended that the bacteria should be present in a concentration of at least 10^7 CFU/mL to the date of consumption (Shah, 2000). To account for recommended amounts of bacteria at the time of consumption, the initial count to be added to the biscuits was 10^9 UFC/mL. The relationship between the optical density at 550 nm and the plate counts (CFU/mL) for *L. plantarum* was previously determined (Bruno, 2009). Besides, protection of beneficial microorganisms is necessary to maintain bioactive concentration while passing through the adverse conditions of the gastrointestinal tract (Shi et al., 2013). In domestic dogs gastric fluid pH ranges from 1.1 to 2 but the food consumed has a buffer capacity of temporally increasing the stomach pH and protecting the bacteria from exposure to extreme values of pH. A great variety in time of exposure treatments with HCl was found in literature (FAO/WHO, 2002; Chen and Subirade, 2006; Yuksel et al., 2000; George and Abraham, 2006). We decided to use 3 h in HCl to conduct the test under more rigorous conditions, and 2 h to simulate the passage through the intestine with phosphate buffer (pH 7.4). In the present work, the composition of the formulation affected the survival of *L. plantarum* against the gastrointestinal system. W biscuits without coating lost 5 orders of magnitude in log CFU/mL and with coating 3.8 orders and WWS biscuits lost 4 orders with or without coating. The last formulation had a protective effect by itself, masking the beneficial effect of the coating observed with wheat biscuits.

4.2. Effect of storage on bacteria survival

For both type of biscuits, a_w values are low enough to provide high stability against water-mediated reactions (BeMiller and Whistler, 2000) and to avoid development of microbes except for certain type of fungi and yeast. The products obtained complied local regulations (CAA, 2004) regarding the water content on a dry basis and a_w before and after storage.

Regarding reduction of viability during storage, the biscuit composition was a factor to consider since W biscuits without coating decreased almost 3 orders of magnitude of log CFU/mL after 20 days storage, while the WWS biscuits showed a difference of approximately 2 orders. The effect of the coating during storage significantly maintained bacterial counts for both type of biscuits. For WWS biscuits, storage revealed the protecting effect of the coating, that was masked by biscuit composition on fresh products. Moreover, it is worth mentioning that all the biscuits showed counts above the recommended value of 10^7 CFU/mL, after 20 days of storage.

Storage conditions did not modify the survival behaviour of *L. plantarum* bacteria after *in vitro* digestion. No differences in the relative reduction of the magnitude order of CFU/mL between just prepared and stored biscuits were found.

With regard to the effect of inulin on the survival of *L. plantarum*, significant effects were only detected in *in vitro* digestion test for W biscuits and no differences were found in storage assays. Related to this, Takemura et al. (2010), while studying the effects of FOS and inulin on the growth and persistence of *L. plantarum*, concluded that it is necessary to take dietary conditions into account to optimize the health-related effects of prebiotics. The addition of inulin could exert benefitial

effects when the bacteria are in the intestine and could favor their development as highlighted in the literature (Reinhart, 1997; Rastall, 2004).

5. Conclusions

The coating contributed to a higher survival of the bacteria during storage and after the passage through the simulated gastrointestinal system. The same effect was observed after 28 days' storage. These coated biscuits with *L. plantarum* are a good alternative as a supplement for domestic dogs since they provide viable probiotic bacteria with healthy benefits.

Conflict of Interest

There are no conflict of interest.

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