Impact of whey protein coating incorporated with *Bifidobacterium* and *Lactobacillus* on sliced ham properties

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**ABSTRACT**

Edible coatings/films with functional ingredients may be a solution to consumers' demands for high-quality food products and an extended shelf-life. The aim of this work was to evaluate the antimicrobial efficiency of edible coatings incorporated with probiotics on sliced ham preservation. Coatings was developed based on whey protein isolates with incorporation of *Bifidobacterium animalis* Bb-12®, *Lactobacillus casei* CBQF – Centro de Biotecnologia e Química Fina, Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital, 172, 4200-374 Porto, Portugal

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1. Introduction

The growing demand for ready-to-eat (RTE) and convenient food products is creating some challenges for the control of food quality and safety. Furthermore, today's consumers are greatly concerned about food value and determining their preferences based on sensory and nutritional characteristics (Norton & Sun, 2008).

Food products, such as sliced ham, that have been processed by heat-treatment are generally recognized as safe (GRAS). Nevertheless, contamination with foodborne pathogens during post-processing, such as peeling, slicing and repackaging, may be the cause of outbreaks of foodborne diseases (Reij, Den Aantrekker, & Force, 2004). On the other hand, deleterious microorganisms growing on the product may cause changes on its quality that may compromise its shelf-life and subsequent consumer acceptance. Such rationale have occasioned the search for new ways to inhibit microbial growth in RTE products, while maintaining their quality, freshness, safety and nutritional value. A new tendency in food technology preservation consists in using active packaging, which is a system where product, packaging and environment interact, and may contribute to enlarge this safety border (Appendini & Hotchkiss, 2002; Biji, Ravishankar, Mohan, & Srinivasa Gopal, 2015).

Antimicrobial packaging is an innovative approach within the framework of the active packaging concept, owing their favorable interaction with the food to the possibility of increasing shelf life and improving safety and even organoleptic properties. This technology has been developed to delay, reduce and/or inhibit the growth of microorganisms on the surface of the foods, thus satisfying the consumer's demands for safe, nutritious, convenient and ready-to-use foods (Biji et al., 2015; Durango et al., 2006; Seol, Lim, Jang, Jo, & Lee, 2009).

Traditionally, the antimicrobial effect of edible coatings has been obtained by incorporating in their structure antimicrobial agents, such as enzymes, polysaccharides, bacteriocins and more recently herbs, spices and essential oils (Appendini & Hotchkiss, 2002; Pranoto, Rakshit, & Salokhe, 2005; Tajkarimi, Ibrahim, & Cliver, 2010). More recently, the possibility of including bacteria capable of producing antimicrobial substances in situ has begun to be explored. The first reports published over the last few years (Concha-Meyer, Schóbitz, Brito, & Fuentes, 2011; Gialamas, Zinoviadou, Biliaderis, & Koutsoumanis, 2010; Leonard et al., 2015; Léonard, Degraeve, Gharsallaoui, Saurel, & Oulahal, 2014; Sánchez-González, Quintero Saavedra, & Chiralt, 2013; Sánchez-González, Quintero Saavedra, & Chiralt, 2014) include only lactic acid bacteria (LAB) with main inhibitory effect upon *L. monocytogenes*; none of these studies have...
envisaged studying the effect of beneficial probiotic bacteria for such purpose (Odila Pereira et al., 2016).

The potential of applying antimicrobial coatings to prevent post-processing-surface contamination of foodborne pathogens on ham products has been demonstrated in a few studies (Lee & Min, 2013; Moradi, Tajik, Razavi Rohani, & Oromiehie, 2011; Santiago-Silva et al., 2009; Theinsathid, Visesanguan, Kruenate, Kingcha, & Keeratipibul, 2012).

Despite the existence of these studies none have attempted the incorporation of probiotic strains capable of generating antimicrobial compounds in situ (Kanmani & Lim, 2013; Odila Pereira et al., 2016; Soukoulis et al., 2014; Tapia et al., 2007). Therefore, this study was the first encompassing the application of whey edible films containing probiotics, to exert antimicrobial effect, against contaminant microorganisms in sliced ham. The incorporation of these microorganisms in coatings/films will be particularly innovative, by combining the antimicrobial effect of the coatings (antimicrobial substances synthesis or by competition in situ) with health benefits (as a carriers of probiotic bacteria).

Both, Lactobacillus spp. and Bifidobacterium spp. produce antimicrobial substances (acids and bactericides) that can help protect food, as well as other health benefits, since many strains therein have been proven as probiotics. Probiotic bacteria are defined as “live microorganisms, that when administered in adequate amounts not less than 10^6 CFU/g (preferentially at least 10^7 CFU/g), confer a health benefit on the host” (FAO/WHO, 2002).

Despite its promising application, the main challenge to the incorporation of multifunctional bacteria into food matrices is ensuring their viability (Madureira, Brandão, Gomes, Pintado, & Malcata, 2011). However, there have been several studies in which this objective has been achieved (Kanmani & Lim, 2013; López de Lacey, López-Caballero, Gómez-Esta, Gómez-Guillén, & Montero, 2012; Soukoulis et al., 2014; Tapia et al., 2007).

Recently, Odila Pereira et al. (2016) reported the successful incorporation of B. animalis Bb-12® and L. casei-01 in whey protein dried edible films reaching high cell viable numbers (10^8 CFU/g film) throughout storage time. Although the results are preliminary evidences, it was demonstrated that the study of bacteria with inhibitory properties incorporated in edible coatings is a promising field and may have great interest; this enables the production of functional coatings for foods to assure a protective antimicrobial environment in situ, working as a carrier for stable and viable microorganisms, endowed with beneficial properties to human intestinal flora.

The employment of this type of whey protein film may be limiting in products where the absence of lactose is a required priority. However, processed meat products use mostly whey protein to modify the overall technological and sensorial characteristics of a meat system such as water holding capacity, fat holding capacity and texture properties (Petracci, Bianchi, Mudalal, & Cavani, 2013), so it was a justified matrix to use.

Thus, the objective of this study was to evaluate the effect of edible whey protein coatings incorporating functional Lactobacillus and Bifidobacterium strains on the quality and safety of packed sliced ham foreseeing extension of shelf-life and a potential carrier for viable probiotic cells and at the same time analyzing the potential to be used as a carrier for viable probiotics.

2. Material and methods

2.1. Bacterial strains, media and growth conditions

Bifidobacterium animalis Bb-12® and Lactobacillus casei-01 kindly donated by Christian Hansen (Denmark) were stored at ~80 °C in de Man–Rogosa–Sharpe (MRS) broth (Biokar Diagnostics, France) supplemented with 30% (v/v) sterile glycerol. The microorganisms were re-activated and pre-cultures were prepared in MRS medium supplemented with filter-sterilized 0.05% (w/v) L-cysteine-HCl (Fluka, St. Gallen, Switzerland) and incubated at 37 °C during 24 h under anaerobic conditions. Subsequently, grown cells were harvested by centrifugation at 4000 rpm for 30 min, at 4 °C. The supernatant was discarded and the pellet was resuspended in a 0.9% (w/v) NaCl sterile solution for posterior incorporation in the coatings.

2.2. Formulation of the coatings

The coatings solutions, one for each probiotic strain, were prepared by dissolving 10% (w/v) whey protein isolate (WPI) powder (Armor Proteins, Saint Brice en Coglés, France) in deionized water, according to Pérez-Gago and Krochta (2002). Glycerol was added at 5% (w/w), as a plasticizer, and the resulting solutions were homogenized for 2 h. Subsequently, the solutions were heated in a water bath at 80 °C, for 20 min and cooled to room temperature.

Afterwards, 5% (w/w) of centrifuged solution of 0.9% NaCl inoculum of either B. animalis Bb-12® or L. casei-01 (15 mL) was added to each 300 mL of film solution to attain a final concentration of 10^6 CFU/mL, as described by Odila Pereira et al. (2016).

2.3. Coating the sliced ham

The ham samples were composed of: poultry meat (turkey breast) (60%), water, starch, salt, dextrose, milk proteins, flavoring, emulsifier (sodium tripolyphosphate), gelling agent (processed Eucheuma seaweed), chloride potassium, vegetable fibers, antioxidants (trisodium citrate and sodium ascorbate), spices, preservative (sodium nitrite), flavor enhancer (monosodium glutamate) and smoke flavor (contains soy and milk (including lactose)). The sliced ham was coated in the production chain of the Primor Charcuteria-Prima, S.A. facility, by slice immersion, for 2 min, in each of the previous WPI probiotic coating solutions. The samples were prepared in three conditions, one without bacteria (control ham), one with B. animalis Bb-12® (Cn0) and one with L. casei-01 (Cn0). Therefore, 21 packages per condition were prepared, containing 3 slices each (in total 63 slices were allocated to each condition).

The excess of liquid in the sliced ham was drained for around 30 s, and thereafter it was packaged (three slices of ham in each package) under usual conditions, i.e. modified atmosphere (70% of nitrogen and 30% of carbon dioxide). Packages were stored at 4 °C for 45 days and at each sampling point (0, 5, 10, 15, 25, 35, 45 days), three packages of each condition were evaluated (total of 21 packages per condition).

2.3.1. Enumeration of bacteria

The viability of incorporated probiotic bacteria was studied in the sliced ham during 45 days of storage at 4 °C. The enumeration of microbial contamination was also monitored during the same period through the determination of total mesophilic aerobic bacteria Staphylococcus spp., Pseudomonas spp., Enterobacteriaceae and yeasts and molds.

At each sampling point (0, 5, 10, 15, 25, 35, 45 days) 10 g of ham slice sample were diluted to 1:10 (w/v) in sterile 1% (w/v) sodium citrate (Merck, Darmstadt, Germany) solution into a Stomacher (Seward, West Sussex, UK) bag and homogenized in a Stomacher 400 Circulator (Seward) for 3 min at 260 rpm. Appropriate sequential 10-fold dilutions were done in sterile peptone water and plated, in triplicate, onto the respective medium and incubated under the preferential conditions for the different microorganisms.

The medium for Lactobacillus was MRS and the plates were incubated under anaerobic conditions in a plastic anaerobic jar with an AnaeroGen sachet (an atmosphere generation system, Oxoid, Basingstoke, England) at 37 °C during 48 h. The medium for Bifidobacterium was MRS supplemented with filter-sterilized 0.05% (w/v) L-cysteine-HCl (Fluka, St. Gallen, Switzerland) and incubated at 37 °C.
during 48 h under anaerobic conditions in a plastic anaerobic jar with an AnaeroGen sachet (an atmosphere generation system, Oxoid, Basingstoke, England). Total mesophilic aerobic bacteria were enumerated on plate count agar (PCA) (Biokar Diagnostics, France). Staphylococcus spp. were enumerated on Baird-Parker agar (BPA) (Lab M, Bury, UK), supplemented with egg-yolk and tellurite emulsion (Biokar Diagnostics, France), as proposed by Baird-Parker (1969) and Pseudomonas spp. were enumerated on Pseudomonas agar base (Lab M, Bury, UK). Plates from PCA, BPA and Pseudomonas media were incubated aerobically at 37 °C for 24 h. Enterobacteriaceae were counted on violet red bile glucose agar (VRBGA) (Lab M, Bury, UK), incubated aerobically at 37 °C for 24 h. The yeasts and molds were determined on rose bengal agar (Lab M, Bury, UK), with 0.1 g/L chloramphenicol (Fluka, Buchs, Switzerland), incubated aerobically at 30 °C for 3 d. The surface plating technique described by Miles, Misra, and Irwin (1938) was followed for all samples and growth media, except with violet red bile glucose agar, for which the pour-plate technique was used.

2.4. Sliced ham characterization

2.4.1. Water activity

The water activity (aw) was measured using a HygroLab 2 (from Rotronic, Basserdorf, Germany). Samples (ca. 0.5 g) were placed on the sample holder; a sealed system was formed by placing the water activity probe on top of the sample holder. The probe was equipped with a small fan to circulate air inside the sample container, a thin film capacitance sensor able to measure RH from 0 to 100 ± 1.5%, and a platinum resistance temperature detector with a precision of ± 0.3 °C. When aw became constant (which usually took < 1 h), its value was recorded. Calibration resorted to six saturated solutions of known a_w (viz. LiCl = 0.114, MgCl_2 = 0.329, K_2CO_3 = 0.443, Mg (NO_3)_2 = 0.536, NaBr = 0.653 and KCl = 0.821). The assays were run in quadruplicate.

2.4.2. Color

To measure the color a portable Chroma meter CR-400 (from Minolta Chroma, Osaka, Japan) with a °C D65 illuminant, with a light source of pulsed xenon lamp, an aperture size of 8 mm, a closed cone and a standard observer of 2° Closely matches CIE 1931 (x_127 = 0.23, y_127 = 0.70), was used. A CIELab color scale was employed to measure the degree of lightness (L), redness (+ a) or greenness (- a), and yellowness (+ b) or blueness (- b) of the films. A white standard plate, with color coordinates L_0 = 97.7, a_0 = 0.04 and b_0 = 1.47, was used to calibrate the equipment. The color of the ham slices was expressed as the total difference in color (ΔE), calculated according to:

\[ ΔE = \left[ (L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2 \right]^{1/2} \]

where L_0, a_0 and b_0 are the initial values obtained for slices of ham under each experimental condition, and L, a and b were the values measured throughout the storage period.

For each condition, four samples were measured – and, on each slice, four readings were made on each side.

2.4.3. Weight loss

Each ham slice was individually weighed on an automatic electrobalance, with a precision of 0.001 g, on the first day, upon coating, and during the storage period at each sampling point. The relative weight loss, ΔW, was calculated as

\[ ΔW = \left( \frac{w_0 - w}{w_0} \right) \times 100 \]

where w_0 is the initial weight and w is the final weight at each sampling point. Three readings of each slice were assessed.

2.4.4. pH measurement

The pH value was measured using a pH meter (Micro pH 2002, Crison, Barcelona, Spain), equipped with a probe for surface in contact directly with the ham sample. The instrument was calibrated prior to and immediately after each session using pH 4 and pH 9 standards as per the manufacturer’s instructions. Three readings of each ham sample were taken.

2.5. Consumer study

The study was conducted in accordance with the Declaration of Helsinki. Participants were informed about the general aim and procedures for handling personal data and gave written informed consent prior to participation. All samples were prepared using food ingredients obtained via commercial suppliers and all additives were food-grade. Preparation prior to testing was performed in a dedicated preparation kitchen and samples were produced and prepared according to good hygiene and manufacturing practices.

Seventeen subjects participated in the consumer test. All participants consumed turkey sliced ham at least once a week and 72% consumed it daily, their age ranged between 24 and 49 years old (31 ± 7) and 59% were female. Overall liking (OL) was evaluated using a 9-point hedonic scale (Gaze et al., 2015; Jones, Peryam, & Thurstone, 1955; Peryam & Pilgrim, 1957). The appropriateness of the intensities of five sensory attributes – brightness, odor, flavor, acid taste, and texture was evaluated by ratings provided on a 5-point, just-about-right scale, where 1 and 2 corresponded to too weak (TW) evaluations, 3 to just-about-right (JAR) and 4 to 5 to too strong (TS) evaluations (Popper, 2014).

Samples (one slice of each ham) were assigned 3-digit codes and were presented to consumers following an incomplete balanced design. No information about the samples was provided to participants, except for safety and hygiene considerations related to their production and preparation. Water was provided to clean the palate between tastings. The tasting sessions took place in the ISO 8589 (ISO, 2007) compliant sensory facilities of Escola Superior de Biotecnologia.

2.6. Statistical analyses

Analysis of variance was performed to determine whether probiotic bacteria (i.e. B. animalis Bb-12® or L. casei-01), coating (presence of bacteria or absence of bacteria) or storage time (0–45 d) were statistically significant sources of variation, at the 0.05 level of significance. Homoscedasticity requirements were met, i.e. experimental errors were independently and normally distributed and possess a constant variance. A paired Tukey’s test was used to test for significant differences in the different microbiological and physico-chemical parameters between packaging systems and controls. The significance level was set at P < 0.05.

All tests were performed to a 5% significance level, using Statistical Package for Social Sciences, v. 17.0 (SPSS, Chicago IL, USA).

Data collected for the sensory analysis was performed with XLSTAT software V. 2015 (Addinsoft, Paris, France).

Overall liking ratings of Cba and Clc were compared pairwise with the control sample using the Friedman’s test. For each sample and for each sensory attribute, the frequencies of intensity ratings (TW, JAR, TS) were determined and the corresponding proportions calculated and compared using z-test. A weighted penalty analysis was conducted to relate attribute intensity ratings to OL (Popper, 2014). Weighted penalties corresponding to < 20% of respondents and to mean drops under 1.0 were considered negligible (Popper, 2014).

3. Results and discussion

The physicochemical and microbiological analyses of sliced ham coated with the previously developed edible formulation (i.e. 10% (w/v) WPI with 5% (w/w) glycerol), as well as, that further incorporated with two probiotic bacteria, throughout 45 days of storage at 4 °C.
3.1. Microbiological characterisation of sliced ham

Enumeration of viable cell numbers was performed during 45 days of storage period under modified atmosphere at 4 °C with a dual purpose: i) to monitor the microbial contamination occurring over a 45 days storage period and the role the incorporated probiotic microorganisms may play thereon; and ii) to assess the viability of the selected probiotic microorganisms throughout storage. Results obtained are shown in Figs. 1, 2, 3 and 4.

Overall, results showed an extension of the shelf-life, at least to 45 days, of the sliced modified atmosphere-packed ham, stored at 4 °C, compared to the uncoated ham stored under modified atmosphere at 4 °C with a dual purpose: i) to monitor the microbial contamination occurring over a 45 days storage period and the role the incorporated probiotic microorganisms may play thereon; and ii) to assess the viability of the selected probiotic microorganisms throughout storage. Results obtained are shown in Figs. 1, 2, 3 and 4.

Fig. 1. Viable cell numbers (Log CFU/g) of Lactobacillus casei-01 and Bifidobacterium animalis Bb12 in uncoated ham (Control ham, ) and coated ham with B. animalis Bb-12® (Cba, ) and coated ham with L. casei-01 (Cc, ) stored under modified atmosphere for 45 days at 4 ± 1 °C. Dashed line ( ) - detection limit of growth (10^2 CFU/g).

Fig. 2. Viable cell numbers (Log CFU/g) of Enterobacteriaceae in uncoated ham (Control ham, ) stored under modified atmosphere for 45 days at 4 ± 1 °C. Dashed line ( ) - detection limit of growth (10^2 CFU/g).

Fig. 3. Viable cell numbers (Log CFU/g) of Staphylococcus spp. in uncoated ham (Control ham, ) stored under modified atmosphere for 45 days at 4 ± 1 °C. Dashed line ( ) - detection limit of growth (10^2 CFU/g).

Fig. 4. Viable cell numbers (Log CFU/g) of total mesophilic aerobic bacteria in uncoated ham (Control ham, ), coated ham with B. animalis Bb-12® (Cba, ) and coated ham with L. casei-01 (Cc, ) stored under modified atmosphere for 45 days at 4 ± 1 °C. Dashed line ( ) - detection limit of growth (10^2 CFU/g).
probiotic cells was improved compared to free cells.

In this study sliced ham was coated with whey protein isolate, and the probiotic viability therein is in agreement with results obtained by Tassou (2016) who used Na-alginate based edible films to deliver probiotic bacteria in a high pressure processed meat product, ensuring viable cells above $10^8$ CFU/g in all sliced ham samples during their shelf life at 4, 8 and 12 °C. Recall that the WPI edible film containing the protective LAB cultures themselves for control against contaminant spoilage by contaminant bacteria such as Enterobacteriaceae and Staphylococcus spp. in refrigerated sliced cooked ham and L. casei-01 stored under modified atmosphere at 4 ± 1 °C with 15 days of storage.

Table 1  
Evolution of weight loss (g), water activity ($a_w$) and pH of uncoated ham (Control ham), coated ham with B. animalis Bb-12® (Cba) and coated ham with L. casei-01 (Clc) stored under modified atmosphere for 45 days at 4 ± 1 °C.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Control</th>
<th>B. animalis Bb-12®</th>
<th>L. casei-01</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 days</td>
<td>14.076 ± 0.007a</td>
<td>0.838 ± 0.001a</td>
<td>6.12 ± 0.01i</td>
</tr>
<tr>
<td>5 days</td>
<td>14.076 ± 0.004a</td>
<td>0.937 ± 0.001a</td>
<td>6.07 ± 0.01b</td>
</tr>
<tr>
<td>15 days</td>
<td>14.076 ± 0.002a</td>
<td>0.937 ± 0.001a</td>
<td>6.08 ± 0.01a</td>
</tr>
<tr>
<td>25 days</td>
<td>14.076 ± 0.005a</td>
<td>0.937 ± 0.001a</td>
<td>6.05 ± 0.02b</td>
</tr>
<tr>
<td>35 days</td>
<td>14.076 ± 0.002a</td>
<td>0.937 ± 0.001a</td>
<td>6.04 ± 0.01b</td>
</tr>
<tr>
<td>45 days</td>
<td>14.076 ± 0.001a</td>
<td>0.937 ± 0.001a</td>
<td>6.03 ± 0.01c</td>
</tr>
</tbody>
</table>

Note: a, b, c, d. *Means ± standard error, within the same columns, labeled with the same letter, do not statistically differ from each other ($P > 0.05$).

Overall, uncoated sliced ham samples revealed a low stability to spoilage by contaminant bacteria such as Enterobacteriaceae and Staphylococcus spp. In fact, viable cell numbers detected upon 15 d of storage increased steadily thereafter (up to 45 days) and were beyond the limit permitted by the Portuguese legislation for sliced ham (Regulamento(CE)n.º1441/2007; Santos et al., 2005). During the same storage period, no detectable growth of these contaminant bacteria was reported for sliced ham coated with our edible WPI film containing Lactobacillus casei-01 or Bifidobacterium animalis Bb-12® demonstrating the importance of our coating in the prevention of spoilage microorganisms. Such observed inhibition may be explained either by the possibility of competition mechanisms between probiotic and spoilage bacteria or eventually via the production of antimicrobial substances by the probiotic strains, such as lactic acid or bacteriocins.

Although most studies deal with control of spoilage microorganisms by metabolic products produced by protective starter cultures (namely bacteriocins), in meat products, such as sliced ham (Enan, 2006; Jofré, Aymerich, & Garriga, 2008; Jofré, Garriga, & Aymerich, 2008; Santiago-Silva et al., 2009), some attention has been given to the use of the protective LAB cultures themselves for control against contaminant and pathogenic microorganisms in meat products (Bredholt et al., 2001; Gao, Li, & Liu, 2015; Jacobsen, Budde, & Koch, 2003; Maragkoudakis et al., 2009). For example, Enan (2006) observed an anti-列表ial effect with a plantaricin producing L. plantarum strain in uncooked and cooked chicken meat. Moreover, similar results were achieved by Yildirim, Yildirim, and Johnson (2007) against L. monocytogenes, when Bifidobacterium bifidum and L. lactis bacteriocins were applied in raw chicken meat under refrigeration conditions. Jofré, Aymerich, and Garriga (2008) and Jofré, Garriga, and Aymerich (2008) used nisin and enterocins A and B in combination with high hydrostatic pressure treatment against Salmonella in refrigerated sliced cooked ham and sausages. Furthermore, Santiago-Silva et al. (2009) demonstrated that pediocin-coated package film reduces Salmonella growth in sliced ham. In all these studies it is the presence of added bacteriocins (metabolic
Color characteristics of uncoated ham (Control ham), coated ham with *B. animalis* Bb-12® (Cba) and coated ham with *L. casei*-01 (Clc) stored under modified atmosphere for 45 days at 4 ± 1 °C, viz. *L*—white), *a*—red), and *b*—yellow)

<table>
<thead>
<tr>
<th></th>
<th>L*</th>
<th><em>a</em></th>
<th><em>b</em></th>
<th>Δ<em>E</em></th>
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<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0 days</td>
<td>73.23 ± 0.01 a</td>
<td>5.31 ± 0.02 a</td>
<td>9.72 ± 0.00 a</td>
<td>26.33 ± 0.00 a</td>
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<tr>
<td>15 days</td>
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<td>9.74 ± 0.00 a</td>
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<td>25 days</td>
<td>72.22 ± 0.01 a</td>
<td>5.27 ± 0.01 a</td>
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<td>26.36 ± 0.00 a</td>
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<tr>
<td>35 days</td>
<td>73.22 ± 0.01 a</td>
<td>5.27 ± 0.01 a</td>
<td>9.67 ± 0.00 a</td>
<td>26.36 ± 0.00 a</td>
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<tr>
<td>45 days</td>
<td>72.20 ± 0.00 a</td>
<td>5.27 ± 0.00 a</td>
<td>9.68 ± 0.02 a</td>
<td>26.36 ± 0.01 a</td>
</tr>
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<td><strong>L. animalis Bb-12®</strong></td>
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<tr>
<td>0 days</td>
<td>73.23 ± 0.01 a</td>
<td>5.29 ± 0.01 a</td>
<td>9.67 ± 0.01 a</td>
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<td>45 days</td>
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<td>5.29 ± 0.01 a</td>
<td>9.69 ± 0.01 a</td>
<td>26.36 ± 0.01 a</td>
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<tr>
<td><strong>L. casei-01</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 days</td>
<td>73.24 ± 0.01 a</td>
<td>5.29 ± 0.01 a</td>
<td>9.70 ± 0.02 a</td>
<td>26.35 ± 0.01 a</td>
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<tr>
<td>15 days</td>
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<td>26.36 ± 0.01 a</td>
</tr>
</tbody>
</table>

Note: *δ* = standard error, within the same column, digits do not statistically differ from each other (*P > 0.05*).

3.2. Physicochemical characterization of sliced ham

Weight loss, water activity and pH of sliced ham and coated sliced ham was monitored throughout the storage period.

The *a*<sub>W</sub> ranged from 0.714 ± 0.003 to 0.838 ± 0.001 (*P < 0.001*) in the control sliced ham and 0.932 ± 0.001 to 0.938 ± 0.001 (*P > 0.05*) in both coated sliced hams.

The pH ranged from 5.80 ± 0.01 to 6.12 ± 0.01 (*P < 0.001*) in the control sliced ham and 6.03 ± 0.01 to 6.08 ± 0.01 (*P > 0.05*) in both coated sliced hams.

These parameters have varied significantly (*P < 0.001*) in the control sliced ham during the 45 days storage period (Table 1), except for pH between 0 and 5 days storage. In the control sliced ham, the observed decrease in water activity is related with weight loss as a consequence of the decrease in water content.

Our results are generally in agreement with the findings of Glass, McDonnell, Rassel, and Zierke (2007) who reported that the results for ham pH and water activity are 6.39 ± 0.02 and 0.967 ± 0.000, respectively and with those presented by Bredholt et al. (2001) who described that the ham had pH and water activity values of approximately 6.2 and 0.97, respectively.

It is noteworthy that the coating protects the sliced ham from water loss and maintained the weight and pH during the storage period.
All samples investigated in this study were manufactured entirely from turkey breasts and were all taken from the same piece of ham, to minimize differences in the visual appearance at the beginning of the study (Fig. 5). In Table 2 are displayed the color characteristics reported for all samples of both uncoated and coated sliced ham. All samples showed values around 73.20 ± 0.00 to 73.24 ± 0.01 for lightness ($L^*$), 5.26 ± 0.01 to 5.31 ± 0.03 for redness ($a^*$) and 9.67 ± 0.01 to 9.72 ± 0.00 for yellowness ($b^*$). The results are in agreement with Iqbal, Sun, and Allen (2013) who obtained values of $L^*$ (70.14–77.60), $a^*$ value (3.49–6.02) and $b^*$ (3.72–9.60) in cooked, pre-sliced turkey hams.

The presence of the edible film with either Lactobacillus casei-01 or Bifidobacterium animalis Bb-12* did not affect significantly ($P > 0.05$) the color of the sliced ham ($L^*$, $a^*$, $b^*$ and $\Delta E$). The latter parameter might be of particular interest, not only because $\Delta E$ values > 3 have been reported as a visual threshold for the distinction of color changes by the human eye, but also because of the potential formation of products with free radical scavenging activities that could potentially protect entrapped bacteria from free radical-driven oxidative damage (Martínez-Cervera, Salvador, Muguerza, Moulay, & Fiszman, 2011).

Slices of ham coated with B. animalis Bb-12* and L. casei-01 solutions exhibited a similar appearance.

### 3.3. Consumer acceptance of sliced ham

A consumer study was performed, as was done by B. A. D. Santos et al. (2015), a consumer acceptability using a 9-point hedonic scale to evaluate OL of the samples and subsequently complemented with the JAR scales to provide insights on improvements. Results of consumer assessments showed that hams coated with B. animalis Bb-12* (7.25 ± 1.13) and with L. casei-01 (7.00 ± 1.37) were liked moderately and were better accepted than the control sample (5.59 ± 2.15) ($P < 0.05$).

Fig. 6 shows the frequencies of TW, JAR and TS intensity ratings for each sample and for all sensory attributes evaluated.

A preponderance of JAR ratings was observed for Cba and Clc for the five attributes evaluated, with their frequencies ranging from 56 to 82%. For the control sample, however, lower proportions of JAR ratings ($P < 0.05$) were observed for odor and flavor intensities, than for Cba, whereas higher proportions of TW ratings for brightness were found ($P < 0.05$). Also for the control sample, lower proportions of JAR ratings than for ham coated with Clc were observed for flavor and texture intensities ($P < 0.05$) and a higher proportions of TW rating for brightness intensities. Moreover, penalty analysis showed only negligible penalties for Cba and Clc for all attributes, except for TW flavor evaluations of the last. For the control sample non negligible penalties were observed relatively to TW evaluations of brightness, odor and flavor intensities.

Since significant differences were found in this study, we should, in the future, increasethe number of consumers and elaborate a sensory profile of the coated samples obtained by Polarized Projective Mapping (PPM), such was done by Horita et al. (2017).
4. Conclusions

Based on reported results, WPI edible coatings may act as a suitable matrix to incorporate probiotic Lactobacillus casei-01 or Bifidobacterium animalis Bb-12® and such coating is easily applied on sliced ham.

Application of a probiotic edible coating to sliced ham enables decreased water and weight loss on the ham surface throughout storage contributing to product freshness. Furthermore, no color changes or differences in pH values, between uncoated and coated slices of ham were detected, assuring the expected quality until 45 days.

The antimicrobial edible coatings successfully inhibited detectable growth of spoilage bacteria, at least up to 45 days of storage, more 28 days than the normal recommended shelf-life.

Furthermore, probiotic bacteria numbers were maintained at high and constant levels of ca. 10⁸ CFU/g throughout storage time, assuring growth of spoilage bacteria, at least up to 45 days of storage, more 28 days than the normal recommended shelf-life.

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References


