Food and Bioprocess Technology (2020) 13:1074–1086
https://doi.org/10.1007/s11947-020-02465-2

ORIGINAL PAPER

Active Whey Protein Edible Films and Coatings Incorporating *Lactobacillus buchneri* for *Penicillium nordicum* Control in Cheese

Ana Guimarães[1] · Óscar Ramos[2] · Miguel Cerqueira[3] · Armando Venâncio[1] · Luís Abrunhosa[1]

Received: 3 February 2020 / Accepted: 14 May 2020 / Published online: 25 May 2020
© Springer Science+Business Media, LLC, part of Springer Nature 2020

Abstract

Fungal contamination of food is responsible for health issues and food waste. In this work, the incorporation of a lactic acid bacteria (LAB) with antifungal properties (*Lactobacillus buchneri* UTAD104) into whey protein-based films and coatings was tested for the control of an ochratoxigenic fungi (*Penicillium nordicum*) in a cheese matrix. The incorporation of *L. buchneri* cells resulted in thicker films with less luminosity than control films and colour alteration. Nevertheless, cells inclusion did not alter moisture content, water vapour permeability, mechanical properties, hydrophobicity and chemical structure of the films. Whey protein films were able to maintain the viability of *L. buchneri* UTAD104 cells in 10^5 CFU/mL after 30 days of storage at 25 °C. When applied in cheese, films and coatings containing *L. buchneri* cells prevented fungal contamination for at least 30 days, while control cheeses with films and coatings either without LAB or with *Lactobacillus casei* UM3 (a strain without antifungal ability) showed fungal contamination during that period. Ochratoxin A was not found in cheeses treated with films and coatings containing *L. buchneri* UTAD104. Results showed that the inclusion of a LAB with antifungal properties in edible films and coatings can help to reduce or eliminate *P. nordicum* contamination in cheeses.

Keywords Bioactive film · Lactic acid bacteria · Antifungal activity · *Penicillium nordicum* · Cheese · Ochratoxin A

Introduction

Moulds are the primary spoilage organism in various food products, such as cereals, bread and fermented dairy products (Schnurer and Magnusson 2005). Cheeses are particularly vulnerable to the growth of filamentous fungi due to their pH, water activity and nutritional profile (Hymery et al. 2014). Fungal contamination can produce discolouration, off-flavours, alterations in texture and appearance, leading to loss of quality, waste and consequently, economic losses. Additionally, some of the spoilage fungi can produce toxic secondary compounds named mycotoxins. These toxins are a serious concern to public health because they cause mutagenic, carcinogenic, cytotoxic, neurotoxic and immunogenic effects, among others (Hymery et al. 2014).

The use of lactic acid bacteria (LAB) in a perspective of biopreservation (inhibition of one microorganism by another) is considered as an alternative to chemical methods for the control of fungal contamination (Reis et al. 2012). LAB present several advantages for this biological strategy. They occur naturally in many foods, have a long history of usage in the manufacture and fermentation of dairy products, are generally regarded as safe (GRAS) and have, in some cases, probiotic properties (Giraffa et al. 2010; Rhee et al. 2011; Varsha and Nampoothiri 2016; Prema et al. 2010). LAB antifungal properties are the result of a synergetic effect of several factors combined, such as the decrease of environmental pH, competitive growth and production of antifungal substances (Guimarães et al. 2018b; Guimarães et al. 2018c; Gerez et al. 2013; Corsetti et al. 1998; Bianchini and Bullerman 2009; Özcelik et al. 2016; Niku-Paavola et al. 1999; Lavermicocca et al. 2000; Ström et al. 2002; Rizzello et al. 2011; Prema et al. 2010). However, in some particular cases, LAB may lose their metabolism and viability, which impairs their ability to exert antifungal effects (Burgain et al. 2011).

✉ Luís Abrunhosa
luisjap@deb.uminho.pt

[1] CEB, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

[2] Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina, Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

[3] INL, International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga, 4715-330 Braga, Portugal

The use of edible films and coatings is a strategy that can overcome this problem since the viability of microorganisms can be protected and maintained above 10^6 CFU/g to keep an adequate concentration of active compounds in the surface of the food products (Soukoulis et al. 2014c).

Edible films and coatings can be competent carriers of LAB, but can also improve foods quality by acting as semipermeable barriers to gases by controlling lipid oxidation, loss of volatile aromas and flavours and by improving the mechanical integrity of the food product (Guimarães et al. 2018a). Also, edible films and coatings meet environmental concerns since they are usually produced with food-grade by-products from agricultural and marine sources (Tharanathan 2003).

Protein-based films and coatings have been gaining interest due to their functional and nutritional properties (Ramos et al. 2016; Ozdemir and Floros 2008; Ramos et al. 2012a). Whey protein is a by-product of cheese production, which represents 20% of the total protein content in milk (Ramos et al. 2012a; Campos et al. 2010). It is a suitable material for the production of edible films and coatings with transparent, flavourless and flexible characteristics, as with good mechanical, oxygen and aroma barrier properties (Pérez-Gago and Krochta 2001; Pérez-Gago et al. 1999; Khwaldia et al. 2004; Ramos et al. 2013; Campos et al. 2010). Previous studies reported that whey protein films, in conjugation with soluble prebiotic fibres, promote the stability of *Lactobacillus rhamnosus* GG (Soukoulis et al. 2014b) and can be effective carriers of this microorganism in pan bread (Soukoulis et al. 2014c). Additionally, several works have combined edible films and coatings with probiotic LAB strains (Tapia et al. 2007; Kanmani and Lim 2013; Piermaria et al. 2015). Regarding the antimicrobial activity of films and coatings incorporating LAB, some studies have shown positive results in the inhibition of spoilage bacteria even if their application in real food conditions is still scarce (Gialamas et al. 2010; Sánchez-González et al. 2013; Sánchez-González et al. 2014; Pereira et al. 2018; Dai et al. 2018; Settier-Ramírez et al. 2019; Ye et al. 2018; Pavli et al. 2017). Nonetheless, to our knowledge, only a few works approached the incorporation of living bacteria in edible films and coatings to control fungal contamination (Pereira et al. 2018; Bambace et al. 2019), and none has evaluated these films in cheese products and their influence in the production of mycotoxin yet.

The present work aimed at investigating the antifungal properties of whey protein-based films containing LAB and at studying the influence of cells addition on the films physicochemical and structural properties. The produced antifungal coatings were also tested in a cheese matrix against *Penicillium nordicum*, and their influence in the production of ochratoxin A (OTA) by this fungus was evaluated.

Materials and Methods

Biological Materials and Growth Conditions

Penicillium nordicum MUM 08.16 (CBS 112573) was revived in yeast extract sucrose (YES) agar in the dark at 25 °C. YES agar formulation consisted in 150 g/L of sucrose, 20 g/L of yeast extract, 15 g/L of agar, 0.5 g/L of $MgSO_4$, 0.01 g/L of $ZnSO_4$ and 0.005 g/L of $CuSO_4$. After 7 days of growth, fungal spores were suspended in a 0.1% peptone solution with 0.05% Tween 80 (Fisher Chemical, USA). The spore concentration of inocula was counted in a microscope using a Neubauer cell chamber. Spore concentration was adjusted to 10^6 spores/mL, and aliquots of spore solutions were stored at − 20 °C during the experimental work. *Lactobacillus buchneri* UTAD104 and *Lactobacillus casei* UM3 were isolated from milk and silage and posteriorly stored in glycerol stocks in our private collection. Strains were molecularly identified by STAB VIDA laboratory (Portugal).

L. buchneri UTAD104 was previously selected for its antifungal ability against *P. nordicum* MUM 08.16 (Guimarães et al. 2018c) and *L. casei* UM3, which showed no antifungal activity (Guimarães et al. 2018b), was used as a negative control. For the production of antifungal films and coatings, LAB were cultivated in MRS broth (Oxoid, UK) supplemented with 20% of tomato juice (Fluka, Switzerland) and 1 g/L of cysteine (Fisher Chemical, USA) at 30 °C for 48 h. A 5% aliquot from the overnight culture was transferred into the 30 mL of modified MRS broth and grown again at 30 °C for 48 h. The bacteria cultures were concentrated by centrifugation (10 min at 7000 rpm), and cells number was adjusted with the MRS culture broth for a concentration of approximately 1×10^{11} CFU/mL.

Production of Films and Coatings

Whey protein concentrate (WPC) (kindly supplied by Armor Proteines, Saint-Brice-en-Coglés, France) was used as the base material for film and coating formulations. Glycerol (87% purity, Panreac, Spain) as a plasticiser, guar gum (Sigma-Aldrich, USA) as a thickener, and Tween 20 (Acros Organic, Belgium) as a surfactant were also used. All the materials, except WPC, were previously sterilized at 121 °C for 15 min.

Film-forming solutions were prepared by adapting the protocol reported by Ramos et al. (2012b). Briefly, WPC (10%, *w*/*w*) was dissolved in distilled water, and then glycerol (5%, *w*/*w*) was added to the solution, which was magnetically stirred for 2 h. The solution was then heated in a water bath at 80 °C under agitation for approximately 5 min. The solution was allowed to cool down at room temperature to approximately 30 °C. Then, Tween 20 (0.2%, *w*/*w*) was added, and the solution was magnetically stirred for another 20 min. After

Springer

this, guar gum (0.7%, *w/w*) was added, and the solution was again magnetically stirred for approximately 16 h. This agitation period was necessary to hydrate the guar gum thoroughly in order to improve the films' homogeneity. Afterwards, film-forming solutions were adjusted to pH 6 using pure glacial acetic acid (Fisher Scientific, USA).

L. buchneri UTAD104 and *L. casei* UM3 were individually added at a concentration of 10, 20 or 30% (*w/w*) to the film-forming solution and homogenized. This corresponds, respectively, to 1×10^{10}, 2×10^{10} and 3×10^{10} CFU/mL in the film solution. Finally, the film-forming solution was poured onto Teflon-coated plates. The same quantity of solution (approximately 1 g for 5 cm^2) was poured onto each Teflon plate to obtain similar film thickness. Film-forming solutions were allowed to dry in an oven with ventilation at 25 °C for 24 h. Once dried, films were peeled off and stored at room temperature in a sealed compartment.

For the antifungal coatings, *L. buchneri* UTAD104 was added at a 30% (*w/w*) and the solution was applied directly onto the cheese surface with the aid of a sterile syringe, to ensure that the entire surface was covered. The residual coating solution was allowed to drip off.

In Vitro Antifungal Activity of Films Against *Penicillium nordicum*

Three sets of films were tested to assess its activity against *P. nordicum*: (i) films incorporating *L. buchneri* UTAD104, (ii) films incorporating *L. casei* UM3 and (iii) films without any LAB (the two last formulations were tested as negative controls). Films antifungal activity was evaluated by the ability of *P. nordicum* to grow on the film surface. Films circles (20 mm of diameter) were placed in Petri plates with YES agar and 10 μL of a spore solution (10^6 spores/mL) of *P. nordicum* was center inoculated in the film's surface. Plates were incubated at 25 °C for 7 days. Visual inspection was conducted daily until 7 days of growth. All experiments were done in duplicate.

Enumeration of Lactic Acid Bacteria in the Films

The viability of incorporated *L. buchneri* UTAD104 and *L. casei* UM3 in the formed films was analysed. Each film was cut into circular discs with 20 mm of diameter (approximately 0.1 g) and placed in a sterile falcon with 9.9 mL of PBS. PBS was prepared in the following way: 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4 and 0.24 g of KH_2PO_4 were added to 1 L of distilled water, and the final pH set to 7.4. The falcons were strongly vortexed until the film was dissolved. Sequential tenfold dilutions were done with PBS and plated on MRS agar. Plates were incubated at 30 °C until colonies were visible. Films were stored in sterile Petri plates sealed with parafilm at room temperature and analysed every 3 to 5 days. Films were analysed until 30 days after casting.

Film Characterization

Physicochemical and mechanical properties of control films without LAB and films incorporating *L. buchneri* UTAD104 at 30% (*w/w*) (films that demonstrate a stronger *P. nordicum* inhibition) were fully characterized as detailed below. Before characterization, films were conditioned at approximately 54 ± 1% relative humidity (RH) and 25 ± 2 °C for at least 24 h.

Thickness

Film thickness was measured with a hand-held digital micrometer (Mitutoyo, Japan) with 0.001 mm accuracy. Thickness was measured randomly at five different points in each sample. Two samples for each film were analysed. Mean values were used for further calculations.

Moisture Content and Water Solubility

Moisture content was determined gravimetrically by drying film samples (discs of 20 mm diameter) at 105 °C for 24 h. Moisture content was given by the percentage of water removed from the initial film sample. For films' water solubility evaluation, film samples were placed in beakers with 40 mL of distilled water, sealed with parafilm and shaken at 60 rpm at 25 °C for 24 h. The undissolved film was recovered and desiccated at 105 °C for 72 h. The non-soluble part of each film was weighted and solubility, expressed as a percentage, was calculated by the equation:

$$Fs(\%) = \left(\frac{Wi - Wf}{Wi}\right) \times 100$$

where *Fs* was the film solubility, W_i was the initial weight of film as dry matter and W_f was the weight of the undissolved film residue. All experiments were performed in triplicate for each film sample, and two samples of each film were tested.

Water Vapour Permeability

Water vapour permeability (WVP) was determined gravimetrically according to the American Society for Testing and Materials (ASTM) E 96-92 method, as described by Cerqueira et al. (2012). Three samples from each film were cut and placed in a permeation cell (a cup with water at 100% RH; 2.337×10^3 Pa vapour pressure at 20 °C). Cups with a diameter of 4 cm and depth of 7 cm were filled with 50 mL of distilled water. The films were mounted with the upper side facing the controlled environment (desiccator with silica gel

0% RH, 0 Pa, 20 °C). The weight loss of the cups was measured at 2 h intervals for a total of 10 h, to determine water-transferred through the samples films. Water vapour transmission rate (WVPR) was calculated by dividing the slope of the linear regression of weight loss versus time by the film area (g/(day m^2)). Afterwards, WVTR was multiplied by film thickness and divided by vapour pressure differential across the sample film to obtain WVP (g mm/(day m^2 kPa)).

Mechanical Properties: Tensile Strength and Elongation at Break

Tensile strength (TS) and elongation at break (EB) were determined with a texture analyser TA.XT Plus (Model TA.HD Plus, Stable Micro Systems, Surrey, UK) according to the ASTM Standard Method D 882-91. Film samples were cut into 120×20 mm^2 strips and placed between the tensile grips. A load cell capacity of 5000 g was used. The initial grip separation and crosshead speed were set to 100 mm and 5 mm/min, respectively. Tensile strength (MPa) was determined by dividing maximum load (N) by the cross-sectional area of the film (m^2), and elongation at break (%) was calculated as a ratio of the final length at the point of sample rupture to the initial length of the sample. At least eight strips of each film sample were analysed.

Optical Properties

The colour of the films was measured using a colorimeter CR 400 (from Konica Minolta, Japan). Colour coordinates L^* (black to white hue component), a^* (red to green hue component) and b^* (yellow to blue hue component) of CIELab colour scale were measured in five random points of each sample, and two samples from each film were tested. Films were measured against the surface of a white standard plate with defined colour coordinates ($L_{\text{standard}} = 97.7$, $a_{\text{standard}} = 0.04$ and $b_{\text{standard}} = 1.47$).

The total colour difference (ΔE) was calculated according to the following equation:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^*, Δa^* and Δb^* are the differentials between the film colour parameter and the colour parameter of a standard used as the film background.

The opacity of each sample was determined according to the Hunterlab method, as a relationship between the opacity of the sample on a black standard and the opacity of the samples in a white standard. Five measurements were made for each sample, and two samples of each film were tested.

Surface Hydrophobicity

Surface hydrophobicity of the films was evaluated by measuring the contact angle of a water droplet with the surface of the film, with an optical contact angle measuring system (OCA20, DataPhysics, Germany) equipped with the C20 software and with CCD video camera (resolution of 752×582 pixel) at 23 °C. Measurements were performed according to sessile drop methods using a 500 μL syringe filled with ultra-pure water. Film samples were tape-glued to the centre of the equipment stand, and a drop of 2 μL was placed on the film surface. Measurements were made from 0 to 60 s, and ten replicates (five from each sample) were analysed for each film type.

Fourier Transform Infrared Spectroscopy

The Fourier transform infrared spectroscopy (FTIR) spectra of the films with and without cells were recorded with a Bruker FT-IR VERTEX 80/80v (Boston, USA) using attenuated total reflectance mode (ATR) with a platinum crystal accessory. Film spectra result from 16 scans in the wavelength range 4000–400 cm^{-1}, at a resolution of 4 cm^{-1}. Before analysis, an open bean background spectrum was recorded as a blank.

X-Ray Diffraction

Crystallinity phases of the sample films were evaluated by X-ray diffraction (XRD). XRD patterns of each film were analysed between $2\theta = 10°$ and $2\theta = 50°$ with a step size $2\theta = 0.02°$ and recorded 14 pts/s with a Cu source, X-ray tube ($\lambda = 1.54056$ Å) at 45 kV and 40 mA in an X-ray diffraction instrument (X'Pert[3] Powder, Panalytical, Almelo, Netherlands).

Antifungal Activity of Films and Coatings Against *Penicillium nordicum* in Cheese

The cheese used for this study was a sliced, semi-hard 'Flamengo' cheese, obtained commercially and manufactured by 'Terra Vento' (São Miguel, Portugal). This cheese is produced with pasteurized bovine milk and packed in a protective atmosphere. It requires refrigeration at low temperature (0 to 10 °C). The typical composition of this cheese is 23.6% of protein, 27.8% of fat and 1.4% of salt. For the following experiments, samples of approximately 4 cm of diameter and 0.5 cm of thickness were used.

Films were placed on top of the cheese surface and centre inoculated with *P. nordicum* (10^6 spores/mL) to determine their antifungal activity. Films incorporating *L. buchneri* UTAD104 and *L. casei* UM3 at 30% (*w*/*w*), and films without cells and untreated cheese were tested in duplicate.

The same WPC solutions were used to directly coat the cheese samples, thus avoiding the film-forming step to simulate better the application conditions of the industry. Cheese samples were left to dry in a vertical flow chamber for 1 h at room temperature. After that period, they were transferred to an incubator with air circulation at 25 °C for approximately 24 h, to ensure that the coating was completely dry. Cheeses samples were then centre inoculated with *P. nordicum* (10^6 spores/mL).

All cheese manipulation, and films and coating application were performed in a sterile environment. After inoculation, cheeses samples were placed in an incubator at 25 °C and approximately 90% RH. The growth of the fungi was assessed every day qualitatively by visual inspection using a magnifying glass over a storage period of 30 days.

Mycotoxin Analysis

Production of OTA in cheeses samples inoculated with *P. nordicum* was evaluated after 30 days of the inoculation. The cheese samples with 4 cm of diameter and approximately 0.5 mm of thickness were cut in small pieces (approx. 1 cm^2) to facilitate the extraction, placed into falcons and extracted with 20 mL of an acetonitrile/methanol/acetic acid (78/20/2, *v*/*v*/*v*) solution. Then, falcons were subjected to vigorous vortexing and placed in the dark at room temperature overnight. Samples were centrifuged (3000 rpm, 10 min), to deposit cheese residues, and supernatants were filtered using a 0.45 μm nylon syringe filters (Whatman, USA) into amber vials and analysed by high-performance liquid chromatography (HPLC) with fluorescence detection as previously reported (Guimarães et al. 2018c). The HPLC system consisted of a Varian Prostar (USA) 210 pump and 410 autosampler, connected to a Jasco (Japan) FP-920 fluorescence detector (λ_{exc} = 333 nm and λ_{em} = 460 nm). A Varian 850-MIB data system was used to establish the interface between the HPLC system and a computer, and the chromatographic data were analysed by Galaxy software. The column used to perform chromatographic separation was a C_{18} reversed-phase YMC-Pack ODS-A analytical column (250 × 4.6 mm I.D., 5 μm) that was protected by a pre-column with the same stationary phase. The mobile phase used to elute OTA was a mixture of water/acetonitrile/acetic acid (99/99/2, *v*/*v*/*v*) that was previously filtered with 0.2 μm GHP membrane filter (Pall, USA) and degassed for 20 min in ultrasounds. The flow rate was set to 1.0 mL/min for a 15 min isocratic run, and the injection volume was 50 μL. Calibration curves were done with a certified OTA standard solution (CRM46912, Sigma-Aldrich, USA) in the mobile phase at concentrations between 0.05 and 100 ng/mL. The concentration of OTA was obtained by comparing peak areas with the calibration curves and limits of detection and quantification for OTA were 0.18 ng/mL and 0.54 ng/mL, respectively.

Data Analysis

At least three independent replicates were performed in all experiments and results were expressed as mean values ± standard deviation. Data were compared by mean analysis (*t* test) at $p < 0.05$ using the software Microsoft Excel version 14.6.0 (Microsoft Corporation, USA).

Results and Discussion

Antifungal Activity of WPC-Based Films

A preliminary assay was used to evaluate the antifungal activity of WPC-based films incorporating LAB. Figure 1 shows the antifungal capacity of the WPC-based films after 7 days of inoculation with a *P. nordicum* spore solution. As expected, the films incorporating the strain *L. casei* UM3 showed no inhibitory effect on *P. nordicum* growth (Fig. 1a, b and c) at all concentrations tested (10, 20 and 30% (*w*/*w*)). On the other hand, *P. nordicum* was not able to grow on the surface of WPC-based films incorporating *L. buchneri* UTAD104, whatever the cell concentration used (Fig. 1d, e and f). Additionally, in an agar diffusion assay, films containing 30% of *L. buchneri* UTAD104 exhibited higher inhibition halos than those incorporated with 10 and 20% of the bacteria (data not shown), thus the 30% concentration was selected for the following studies.

The antifungal capacity of *L. buchneri* UTAD104 has been reported previously and associated with the production of acetic, lactic and phenyllactic acids (Guimarães et al. 2018c). These organic acids already demonstrated antifungal ability in other studies (Prema et al. 2010). In films, the produced organic acids are probably inhibiting *P. nordicum* growth. This is possibly the primary inhibitory mechanism involved, since others, such as the competitive growth, would also produce an inhibitory effect in films incorporating the strain *L. casei* UM3. On the contrary, films incorporated with *L. casei* UM3 did not show antifungal activity.

Lactic Acid Bacteria Survival in Films

Viability of *L. buchneri* UTAD104 and *L. casei* UM3 incorporated in WPC-based films was assessed throughout a storage period of 30 days at room temperature in order to evaluate films capacity to maintain cell survival. Counts of viable LAB over that period are shown in Fig. 2.

For both strains, there is a consistent decrease in the number of viable cells in the films throughout the 30 days of storage. In the first 15 days, there was a more accentuated decrease in viability, of approximately 5 log CFU/mL. After that period and until 30 days of storage, the decrease in viable LAB was less accentuated (approximately 2 to 3 log).

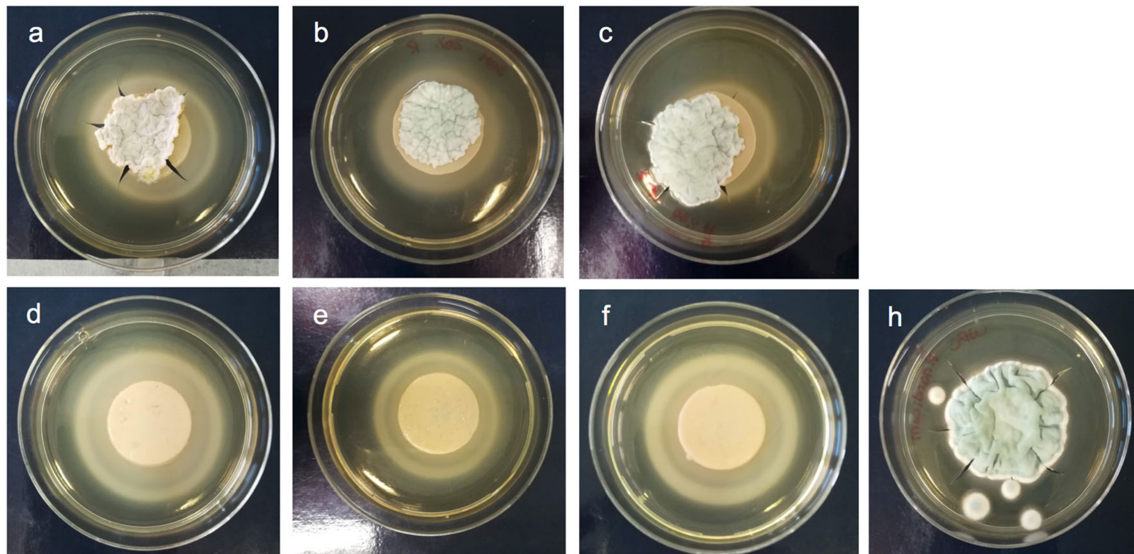

Fig. 1 Antifungal activity of WPC based films against *P. nordicum* by inoculation of a spore solution in the surface of the films. Films incorporating *L. casei* UM3 at 10% (**a**), 20% (**b**) and 30% (*w*/*w*) concentration (**c**); films incorporating *L. buchneri* UTAD104 at 10% (**d**), 20% (**e**) and 30% (*w*/*w*) concentration (**f**); samples without film—*P. nordicum* control (g)

Between day 22 and 30, the number of viable cells stabilized around 1×10^5 CFU/mL for both LAB.

In edible films, the presence of relatively high amounts of solutes combined with their rubbery physical state leads to the occurrence of enzymatic and chemical reactions, which can cause damage in essential cellular structures (Soukoulis et al. 2014b), and consequently affect cell survival within those films. Still, nutrients present in the films and in the culture media that were added together with the cells can, usually, support cell viability (Soukoulis et al. 2014a). Furthermore, whey proteins and glycerol are described to have a positive effect on LAB viability (Mohammadi et al. 2011). However, reduction of viable LAB throughout time can still occur due to film dehydration, temperature, presence of oxygen and further depletion of nutrients (Pereira et al. 2016; Sánchez-González et al. 2014).

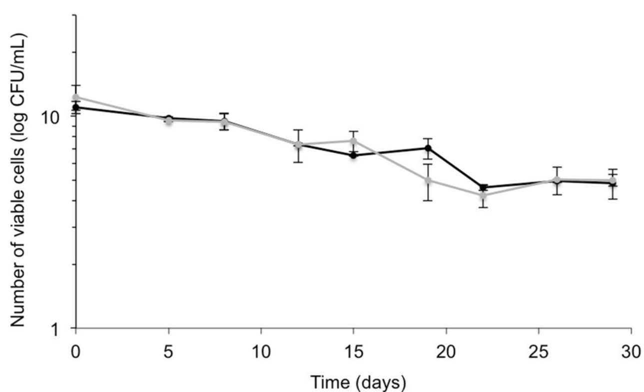

Fig. 2 Number of viable LAB (expressed in log CFU/mL) in the films over time (days). *L. buchneri* UTAD104 represented by the black line and *L. casei* UM3 represented by the grey line

Similar results for cell survival in films were obtained by Fan et al. (2009), which reported the capability of sodium alginate films to maintain half of the *Cryptococcus laurentii* population after a 20-day storage period. Sánchez-González et al. (2013) also reported the ability of films made of pea protein, methylcellulose and sodium caseinate, to maintain at least half of the initial viable counts of *L. plantarum*, after 30 days of storage at 5 °C. Differences in cell survival in film matrix may be attributed to the type of entrapped strain, composition of the film and drying and storage conditions.

Film Characterization

Thickness, Moisture Content, Water Solubility and Water Vapour Permeability

Table 1 shows the values obtained for thickness, moisture content, water solubility and WVP of films with and without the incorporation of *L. buchneri* UTAD104 (30% *w*/*w*). In general, incorporation of LAB did not affect significantly ($p < 0.05$) the moisture content and WVP values in comparison to the plain WPC films (control films). However, the addition of cells decreased the thickness of the films significantly and slightly increased their solubility in water (Table 1).

The thickness of the WPC-based films was significantly affected by the incorporation of cells with values decreasing from 0.23 mm (control film) to 0.17 mm (film incorporating *L. buchneri* UTAD104). The thickness of the obtained control films was, in general, higher than those reported by Pérez-Gago et al. (1999), Ramos et al. (2013) and Pérez et al. (2016) for similar whey protein films, i.e. 0.115, 0.170 and 0.126 mm, respectively. Still, other works reported thicker

Table 1 Thickness, moisture content (MC), water solubility (WS), water vapour permeability (WVP), tensile strength (TS) and elongation at break (EB) of films without and with *L. buchneri* UTAD104

WPC film	Thickness (mm)	MC (%)	WS (%)	WVP (g mm/(day m^2 kPa))	TS (MPa)	EB (%)
Without LAB	0.23 ± 0.02a	26.2 ± 0.9a	43.7 ± 1.6a	9.6a	0.6 ± 0.06a	53.8 ± 25.1a
With *L. buchneri*	0.17 ± 0.03b	25.0 ± 1.2a	45.1 ± 0.9b	10.0a	0.5 ± 0.11a	48.1 ± 21.3a

Different letters within the same column indicate significant differences ($p < 0.05$)

whey protein films (0.31 mm) (Bahram et al. 2014) and others reported similar values (0.229 mm) to those obtained in this work (Reinoso et al. 2008). These differences in film thickness are probably due to different film formulations and film making procedures, even though examples given are whey protein-based.

The moisture content of edible films is an important parameter to estimate other film properties, such as films dissolution ability during human consumption and their capacity to withstand probiotic survival (Kanmani and Lim 2013). The incorporation of cells has not affected the moisture content of the films significantly (25.0 ± 1.2% and 26.2 ± 0.9% for control and *L. buchneri* films, respectively). Similar values of moisture content (27.9%) for WPC-based films with glycerol as a plasticiser were obtained by other authors (Pérez et al. 2016).

Both films (with and without cells) swelled and maintained their integrity during the dissolution procedure for 24 h. Control films presented solubility in water of 43.7 ± 1.6%, while films incorporating cells showed a significantly higher ($p < 0.05$) solubility of 45.1 ± 0.9% (Table 1). The solubility of the control films was relatively similar to other films in the literature (Ramos et al. 2012c; Pérez-Gago et al. 1999; Pérez et al. 2016). The partial insolubility of the films can be attributed to strong intermolecular bonds existing between the protein molecules within the film matrix (Ramos et al. 2013). The higher content of water that resulted from the incorporation of the cell culture can explain the slightly higher solubility of films containing *L. buchneri* UTAD104. It may also indicate lower cohesion of the film matrix, due to the presence of cells.

The WVP is a critical characteristic of edible films once low WVP is generally necessary for the control of detrimental reactions (such as lipid oxidation, browning and vitamin reactions) and the structural decay, which can facilitate microbial spoilage and result in a weight loss of the food product. The incorporation of cells in the film did not significantly alter the WVP of the films, with the WVP of control films and films with *L. buchneri* UTAD104 being 9.6 and 10.0 g mm/(day m^2 kPa), respectively. For the control films, similar WVP were obtained by Ramos et al. (2012c). Alike, Aloui et al. (2015) and Gialamas et al. (2010) reported that the incorporation of cells did not affect the barrier properties of edible films significantly. In contrast, Sánchez-González et al. (2013) reported that the incorporation of *L. plantarum* increased WVP due to the discontinuities introduced in the film matrix.

Mechanical Properties

Edible films should be able to maintain their integrity during processing, storage, shipping and handling. To do so, they must possess adequate tensile strength and elongation (Falguera et al. 2011). Table 1 presents the results of the tensile strength and elongation at break for control films and films with *L. buchneri* UTAD104. Tensile strength indicates the maximum stress that the films sustain during stretching, while elongation at break indicates the increase in film length from the initial point to the breakpoint, giving a measure of the film extensibility.

In general, the results indicate that the presence of cells did not significantly influence the mechanical properties of the films, which indicate that when incorporated in the WPC films, LAB do not interfere in the structure of the proteinaceous network. This is in agreement with results obtained by other authors (Soukoulis et al. 2016; Kanmani and Lim 2013; Gialamas et al. 2010). So, films incorporating *L. buchneri* UTAD104 should be able to stand the same procedures as control films. Obtained values for tensile strength in whey protein films are similar to those observed by Ramos et al. (2012c) and Bahram et al. (2014) (around 0.7 and 0.8 MPa, respectively); however, these values are relatively low when compared with other reported by Pérez-Gago et al. (1999), Reinoso et al. (2008) and Aziz and Almasi (2018) with values around 6.5, 6.1 and 7.1 MPa, respectively. It is known that the content of plasticiser can influence the tensile strength of films, with high contents of plasticiser (such as the used in this work) resulting in low tensile strength values (Fang et al. 2002). Both produced WPC films had an elongation at break of almost 50%, which indicate that films have high extensibility. These values are similar to the one reported by Pérez-Gago (1999) for other whey protein films (43%), even though these films consisted of only 5% WPC. Other authors using 10% of WPI reported a considerably lower elongation at break (19.5%) (Reinoso et al. 2008).

Optical Properties

Colour and optical properties are important characteristics of edible films since they can directly influence consumer acceptance. Colour of the films is affected by several factors that include plasticiser addition, thermal treatments, production process and storage conditions and, in the case of protein films, the protein concentration (Su et al. 2012). The hue values and opacity for both films are shown in Table 2. All analysed parameters were significantly affected by the incorporation of cells, and all samples showed a yellowish colour typical of WPC-based films, which can be linked to the presence of fat and phospholipids (Pérez et al. 2016; Ramos et al. 2013). In general, films incorporating *L. buchneri* UTAD104 have less luminosity (L^*) than control films (90.6 and 94.1, respectively); have a slightly red hue opposed to control films that show a more 'neutral' hue (a^* values of − 0.4 for control films and 0.8 for films incorporating LAB); and are also more yellowish than control films (b^* value of − 22.4 for films with LAB higher than − 10.5 of control film). The addition of *L. buchneri* UTAD104 was also related to an increase in the opacity of the films when compared to control films (13.4 and 12.6%, respectively). The total colour difference (ΔE) that includes the three colour parameters was also calculated for both films. Values of 9.2 and 21.6 were obtained for films without and with *L. buchneri* UTAD104, respectively. This difference confirms that addition of cells alters the colour and optical properties of WPC-based films and that the difference can be perceived clearly, as 3 is considered the threshold of human-perceivable colour differences for ΔE values (Mokrzycki and Tatol 2011). The incorporation of cells within MRS culture media (that presents a brownish colour) is probably the main responsible for these differences of colour.

Surface Hydrophobicity

Water contact angle measurement is used to determine the hydrophilicity ($\theta < 65°$) or hydrophobicity ($\theta > 65°$) of the film surface. This parameter can be used to estimate the resistance of the film to moisture transfer (Phan et al. 2005). The initial contact angle (time 0 s) and contact angles after 1, 5, 15, 30, 45 and 60 s of applied water droplets were measured. The upper side of the films was analysed, and the obtained values are shown in Table 3. In general, both films demonstrated a hydrophilic character ($\theta < 65°$) at 0 s, with similar values of θ for control films and films with *L. buchneri* UTAD104 (51.06° and 51.13°, respectively). These θ values are similar to the ones obtained in other works (Kokoszka et al. 2010). Throughout the experiments, contact angles values dropped until reaching 29.5° and 20.8°, for films without and with *L. buchneri* UTAD104, respectively. The control films showed lower θ values until 30 s after the water droplet application; however, the differences were only statistically significant after 60 s. With time, the swelling of the films (more visible in films with *L. buchneri* UTAD104) and the absorption of the water droplet was noticeable, being observed for times larger than 30 s.

Table 3 Measurements of contact angle (θ) in function of time (s) after water droplet application on films with and without cells of *L. buchneri* UTAD104

Contact angle (θ)		
Time (s)	Film without LAB	Film with *L. buchneri*
0	51.1 ± 2.86 a	51.1 ± 4.78 a
1	40.5 ± 2.24 a	41.1 ± 1.96 a
5	35.7 ± 2.17 a	36.6 ± 1.27 a
15	32.6 ± 2.23 a	34.2 ± 1.14 a
30	30.7 ± 1.69 a	31.7 ± 1.36 a
45	29.9 ± 1.47 a	25.8 ± 7.41 a
60	29.5 ± 1.39 a	20.8 ± 10.35 b

Different letters within the same line indicate significant differences between film formulations ($p < 0.05$)

Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectrum of films was analysed in the region of 4000 to 400 cm^{-1} (Fig. 3a). Similar peaks can be observed for both samples, with and without *L. buchneri* UTAD104, thus indicating that the presence of the bacteria did not alter the chemical structure of the films. The strong absorption band at 3271 cm^{-1} is associated with the stretching vibration of free and bound OH and N-H groups, which can be intensified by the addition of glycerol (Ballesteros et al. 2018;

Table 2 Colour parameters L^*, a^* and b^* and opacity of WPC-based films with and without *L. buchneri* UTAD104

WPC film	L^*	a^*	b^*	Opacity (%)
Without LAB	94.1 ± 0.45a	−0.4 ± 0.03a	10.5 ± 0.33a	12.6 ± 0.30a
With *L. buchneri*	90.6 ± 0.65b	0.8 ± 0.56b	22.4 ± 0.79b	13.4 ± 0.24b

Different letters within the same column indicate significant differences between film formulations ($p < 0.05$)

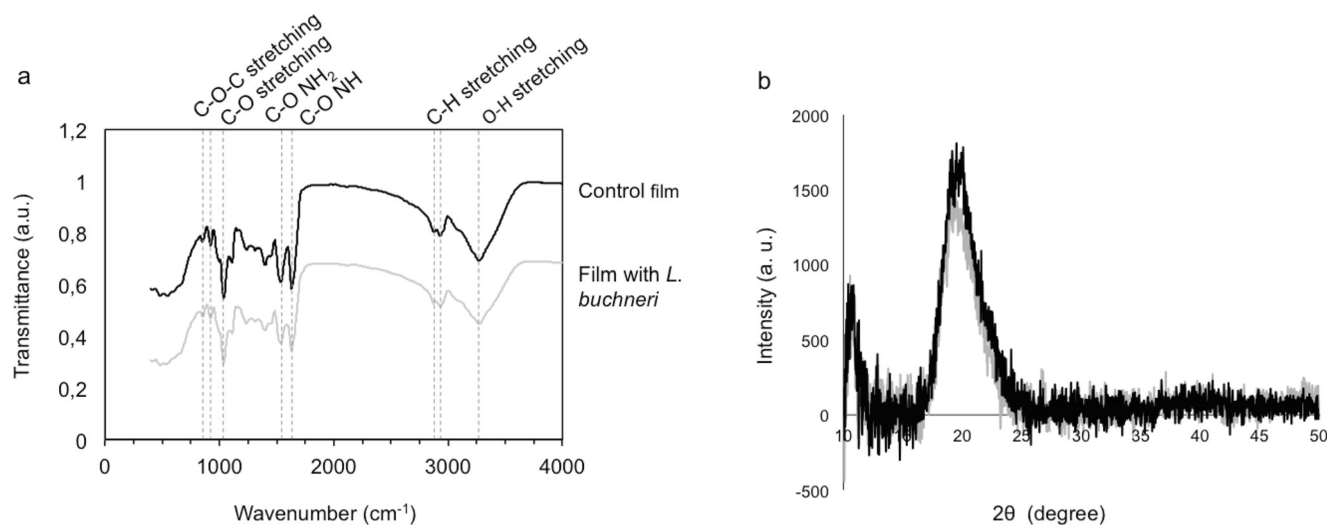

Fig. 3 FTIR (**a**) and X-ray diffraction pattern (**b**) obtained from films without (black) and with *L. buchneri* UTAD104 (grey)

Cerqueira et al. 2012). Literature indicates that the N-H band generally appears at 3253 cm^{-1} (Ramos et al. 2013). So, the presence of the other film components, in particular, glycerol, may be responsible for the band shift seen in the obtained spectra. This band is also characteristic of moist materials. The bands at 2874 cm^{-1} and 2930 cm^{-1} correspond to stretching of –C-H groups. Bands at 1535 cm^{-1} and 1628 cm^{-1} are characteristic of the primary amine group of proteins (-CO-NH_2) and the secondary amide group of proteins (-CO-NH), and both are characteristic of whey proteins. The band around 1035 cm^{-1} corresponds to the C-O band stretching and can be related to the addition of glycerol (López-Rubio and Lagaron 2012; Pérez et al. 2016). Also, bands at 852 cm^{-1} and 924 cm^{-1} correspond to asymmetric and symmetric stretching vibrations of alkoxyl groups (C-O-C) and are also originated by the presence of glycerol in the films (Cerqueira et al. 2012).

X-Ray Diffraction

The X-ray diffraction pattern was used to perceive if the addition of cells to the film caused alterations in the film crystallinity. Generally, in a crystalline material spectrum, several sharp peaks are present, while in amorphous substances, a broad background pattern is shown (Fernandes et al. 2014). XRD spectra of the analysed films (with and without *L. buchneri* UTAD104) showed similar profiles (Fig. 3b). As expected, both films show a non-crystalline structure, presenting two broad peaks at approximately $2\theta = 11°$ and $2\theta = 20°$. This second peak is present in the three raw materials (whey protein, glycerol and guar gum) that compose the tested films and is typical for amorphous structures (Martins et al. 2012), while $2\theta = 11°$ is typically present in whey protein XRD pattern (Le Tien et al. 2000; Li et al. 2011).

Antifungal Activity of the Bioactive Films and Coatings Against *Penicillium nordicum* in Cheese

The effect of the tested WPC-based films on fungal decay of cheese during their storage at 25 °C and 90% RH are presented in Fig. 4. After 30 days of storage, the total absence of fungal growth was only observed on cheese covered with films incorporating *L. buchneri* UTAD104 (Fig. 4d). In control films (without LAB and with *L. casei* UM3), the fungal colonies became visible with a delay of 2 days comparing to uncovered cheeses (data not shown). After 20 days of incubation, their surface was already entirely covered by *P. nordicum* (Fig. 4b and c). For the uncovered cheeses, this occurred soon after 7 days of incubation (Fig. 4a).

Afterwards, the WPC solutions were used to coat the cheese samples directly, eliminating the film-forming step, thus simplifying the application process. Coated cheese samples were stored similarly at 25 °C and 90% RH. After 5 days of storage, the uncoated cheese samples (Fig. 5a) were considerably infected by *P. nordicum*, with the fungi covering all the surface of the cheese sample. Cheeses coated with WPC without LAB and coated with WPC incorporating *L. casei* UM3 (Fig. 5b and c) also developed fungal contamination. However, when compared to uncoated samples, there was a considerable delay in the infection process. Thus, it seems the coating alone affected the development of the fungus (Fig. 5b), probably, because it created a barrier between the fungus and the nutritive cheese surface. Cheese samples coated with WPC incorporating the antifungal strain *L. buchneri* UTAD104 remained free of fungal contamination for the 30-day test period (Fig. 5d). Other authors reported similar results for different coatings materials. One work showed that 'Coalho' cheese coated with alginate/lysozyme nanolaminate coatings had no apparent microbiological contamination after 20 days, while uncoated cheeses showed evident

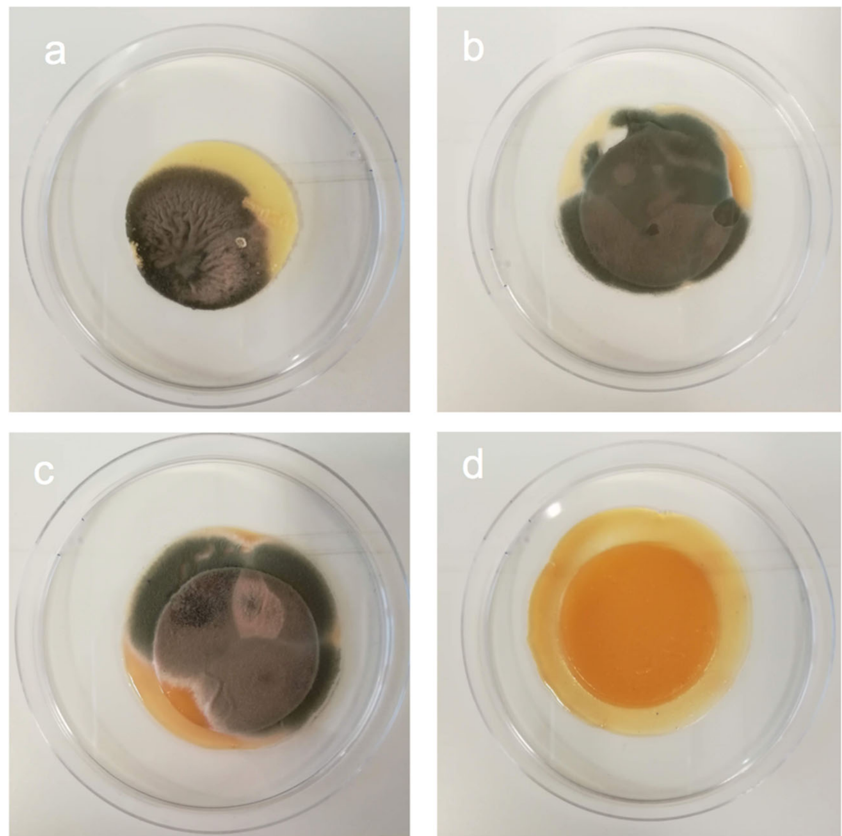

Fig. 4 Growth of *P. nordicum* after 30 days in uncovered cheese (**a**); cheese covered with WPC based film (**b**); cheese covered with WPC-based film incorporating *L. casei* UM3 at 30% (*w*/*w*) (**c**); cheese covered with WPC-based film incorporating *L. buchneri* UTAD104 at 30% (*w*/*w*) (**d**)

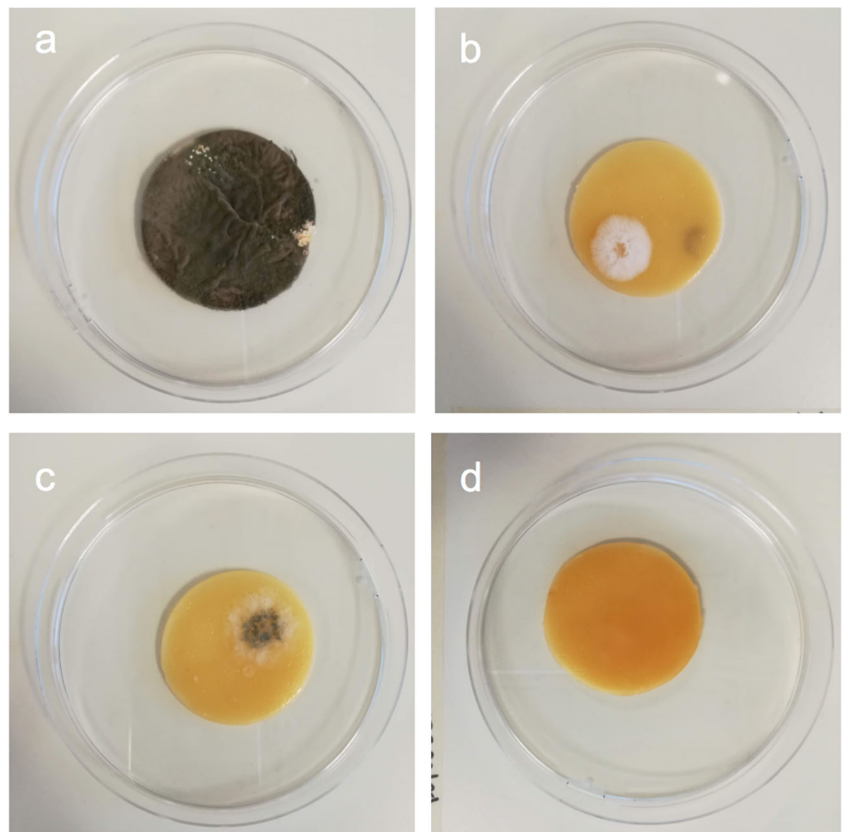

Fig. 5 Growth of *P. nordicum* after 30 days in uncoated cheese (**a**); cheese coated with WPC-based solution (**b**); cheese coated with WPC incorporating *L. casei* UM3 at 30% (*w*/*w*) (**c**); cheese coated with WPC incorporating *L. buchneri* UTAD104 at 30% (*w*/*w*) (**d**)

Table 4 OTA (ng/mL) detected on cheese samples covered with WPC based films and coatings

Cheese	Films		Coatings	
	OTA (ng/mL)	Reduction (%)	OTA (ng/mL)	Reduction (%)
Uncoated	7.5 ± 1.7	–	8.8 ± 1.5	–
WPC film	1.3 ± 0.3	83	0	100
WPC + *L. casei*	0.4 ± 0.3	94	0	100
WPC + *L. buchneri*	0.0 ± 0.0	100	0	100

fungal proliferation (Medeiros et al. 2014). Another work showed that chitosan coatings reduced infection in strawberries up to 3 days of storage when compared with non-coated ones (Perdones et al. 2012). On the other hand, another study presented that sodium alginate and locust bean gum coatings delayed infection in oranges for 5 days when compared with uncoated fruits (Aloui et al. 2015).

When comparing both application strategies, the *P. nordicum* growth was less accentuated in coated cheese samples (Fig. 5) than in film covered ones (Fig. 4). The drying step required after the coating of the cheese can be responsible for this effect, since it lowered samples a_w, thus making fungal germination and colonization more difficult.

Mycotoxin Analysis in Cheese Covered with Films and Coated

OTA content in cheeses samples is shown in Table 4. OTA in cheese covered with WPC-based films was 83% inferior to the uncoated control, while those covered with films containing *L. casei* UM3 showed a reduction of 94% of OTA when compared with the uncoated control. In the cheese samples covered with WPC containing *L. buchneri* UTAD104, no OTA was detected. Since there was no fungal growth in those samples, this was expected. In the case of coated cheeses, OTA was only detected in uncoated samples. The absence (Fig. 5d) and weak growth of *P. nordicum* (Fig. 5b and c) in these coats justify the absence of OTA in those samples. To our knowledge, none of the studies regarding the incorporation of LAB in edible films analysed the mycotoxin content of the product.

Conclusion

This study shows that WPC-based films incorporating a LAB with antifungal properties could potentially be used in food packaging, contributing to an increased shelf-life of cheese. Moreover, it is demonstrated that the presence of cells did not significantly affect some essential characteristics of the films such as moisture content, WVP and mechanical properties. However, the presence of cells had an impact on the thickness and colour of the films. Application of the film with the antifungal LAB strain to cheeses allowed the inhibition of *P. nordicum* growth for a storage period over 30 days, in which 1×10^5 CFU/mL of LAB were still viable. OTA amounts were substantially lower in cheese protected by the WPC based films and coatings.

Acknowledgements This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte. Ana Guimarães received support through grant SFRH/BD/103245/2014 from the Portuguese FCT.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

Aloui, H., Licciardello, F., Khwaldia, K., Hamdi, M., & Restuccia, C. (2015). Physical properties and antifungal activity of bioactive films containing *Wickerhamomyces anomalus* killer yeast and their application for preservation of oranges and control of postharvest green mold caused by *Penicillium digitatum*. *International Journal of Food Microbiology, 200*, 22–30.

Aziz, S. G.-G., & Almasi, H. (2018). Physical characteristics, release properties, and antioxidant and antimicrobial activities of whey protein isolate films incorporated with thyme (Thymus vulgaris L.) extract-loaded nanoliposomes. *Food and Bioprocess Technology, 11*(8), 1552–1565.

Bahram, S., Rezaei, M., Soltani, M., Kamali, A., Ojagh, S. M., & Abdollahi, M. (2014). Whey protein concentrate edible film activated with cinnamon essential oil. *Journal of Food Processing and Preservation, 38*(3), 1251–1258.

Ballesteros, L. F., Cerqueira, M. A., Teixeira, J. A., & Mussatto, S. I. (2018). Production and physicochemical properties of carboxymethyl cellulose films enriched with spent coffee grounds polysaccharides. *International Journal of Biological Macromolecules, 106*, 647–655.

Bambace, M. F., Alvarez, M. V., & Moreira, M. D. R. (2019). Novel functional blueberries: fructo-oligosaccharides and probiotic lactobacilli incorporated into alginate edible coatings. *Food Research International, 122*, 653–660.

Bianchini, A., & Bullerman, L. B. (2009). Biological control of molds and mycotoxins in foods. In Appell, M., Kendra, D. F.& Trucksess, M. W. (Eds.), *Mycotoxin Prevention and Control in Agriculture*

(Vol. 1031, pp. 1–16, ACS symposium series). Washington: ACS symposium series, American Chemical Society.

Burgain, J., Gaiani, C., Linder, M., & Scher, J. (2011). Encapsulation of probiotic living cells from laboratory scale to industrial applications. *Journal of Food Engineering, 104*(4), 467–483.

Campos, C. A., Gerschenson, L. N., & Flores, S. K. (2010). Development of edible films and coatings with antimicrobial activity. *Food and Bioprocess Technology, 4*(6), 849–875.

Cerqueira, M. A., Souza, B. W. S., Teixeira, J. A., & Vicente, A. A. (2012). Effect of glycerol and corn oil on physicochemical properties of polysaccharide films—a comparative study. *Food Hydrocolloids, 27*(1), 175–184.

Corsetti, A., Gobbetti, M., Rossi, J., & Damiani, P. (1998). Antimould activity of sourdough lactic acid bacteria: identification of a mixture of organic acids produced by *Lactobacillus sanfrancisco* CB1. *Applied Microbiology and Biotechnology, 50*(2), 253–256.

Dai, L., Yuan, Y., Song, Z., Qiu, Y., & Yue, T. (2018). Preparation and characterization of *Lactobacilli*-loaded composite films with sustaining antipathogenic activity and preservation effect. *Journal of Food Science, 83*(10), 2511–2519.

Falguera, V., Quintero, J. P., Jiménez, A., Muñoz, J. A., & Ibarz, A. (2011). Edible films and coatings: structures, active functions and trends in their use. *Trends in Food Science & Technology, 22*(6), 292–303.

Fan, Y., Xu, Y., Wang, D., Zhang, L., Sun, J., Sun, L., & Zhang, B. (2009). Effect of alginate coating combined with yeast antagonist on strawberry (*Fragaria × ananassa*) preservation quality. *Postharvest Biology and Technology, 53*(1–2), 84–90.

Fang, Y., Tung, M. A., Britt, I. J., Yada, S., & Dalgleish, D. G. (2002). Tensile and barrier properties of edible films made from whey proteins. *Journal of Food Science, 67*(1), 188–193.

Fernandes, R. V. B., Borges, S. V., Botrel, D. A., & Oliveira, C. R. (2014). Physical and chemical properties of encapsulated rosemary essential oil by spray drying using whey protein–inulin blends as carriers. *International Journal of Food Science and Technology, 49*(6), 1522–1529.

Gerez, C. L., Torres, M. J., Font de Valdez, G., & Rollán, G. (2013). Control of spoilage fungi by lactic acid bacteria. *Biological Control, 64*(3), 231–237.

Gialamas, H., Zinoviadou, K. G., Biliaderis, C. G., & Koutsoumanis, K. P. (2010). Development of a novel bioactive packaging based on the incorporation of *Lactobacillus sakei* into sodium-caseinate films for controlling *Listeria monocytogenes*. *Food Research International, 43*(10), 2402–2408.

Giraffa, G., Chanishvili, N., & Widyastuti, Y. (2010). Importance of lactobacilli in food and feed biotechnology. *Research in Microbiology, 161*(6), 480–487.

Guimarães, A., Abrunhosa, L., Pastrana, L. M., & Cerqueira, M. A. (2018a). Edible films and coatings as carriers of living microorganisms: a new strategy towards biopreservation and healthier foods. *Comprehensive Reviews in Food Science and Food Safety, 17*(3), 594–614.

Guimarães, A., Santiago, A., Teixeira, J. A., Venâncio, A., & Abrunhosa, L. (2018b). Anti-aflatoxigenic effect of organic acids produced by *Lactobacillus plantarum*. *International Journal of Food Microbiology, 264*, 31–38.

Guimarães, A., Venancio, A., & Abrunhosa, L. (2018c). Antifungal effect of organic acids from lactic acid bacteria on *Penicillium nordicum*. *Food Additives & Contaminants: Part A, 35*(9), 1803–1818.

Hymery, N., Vasseur, V., Coton, M., Mounier, J., Jany, J.-L., Barbier, G., & Coton, E. (2014). Filamentous fungi and mycotoxins in cheese: a review. *Comprehensive Reviews in Food Science and Food Safety, 13*(4), 437–456.

Kanmani, P., & Lim, S. T. (2013). Development and characterization of novel probiotic-residing pullulan/starch edible films. *Food Chemistry, 141*(2), 1041–1049.

Khwaldia, K., Perez, C., Banon, S., Desobry, S., & Hardy, J. (2004). Milk proteins for edible films and coatings. *Critical Reviews in Food Science and Nutrition, 44*(4), 239–251.

Kokoszka, S., Debeaufort, F., Lenart, A., & Voilley, A. (2010). Water vapour permeability, thermal and wetting properties of whey protein isolate based edible films. *International Dairy Journal, 20*(1), 53–60.

Lavermicocca, P., Valerio, F., Evidente, A., Lazzaroni, S., Corsetti, A., & Gobbetti, M. (2000). Purification and characterization of novel antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21B. *Applied and Environmental Microbiology, 66*(9), 4084–4090.

Le Tien, C., Letendre, M., Ispas-Szabo, P., Mateescu, M. A., Delmas-Patterson, G., Yu, H. L., et al. (2000). Development of biodegradable films from whey proteins by cross-linking and entrapment in cellulose. *Journal of Agricultural and Food Chemistry, 48*(11), 5566–5575.

Li, Y., Jiang, Y., Liu, F., Ren, F., Zhao, G., & Leng, X. (2011). Fabrication and characterization of TiO2/whey protein isolate nanocomposite film. *Food Hydrocolloids, 25*(5), 1098–1104.

López-Rubio, A., & Lagaron, J. M. (2012). Whey protein capsules obtained through electrospraying for the encapsulation of bioactives. *Innovative Food Science and Emerging Technologies, 13*, 200–206.

Martins, J. T., Cerqueira, M. A., Bourbon, A. I., Pinheiro, A. C., Souza, B. W. S., & Vicente, A. A. (2012). Synergistic effects between κ-carrageenan and locust bean gum on physicochemical properties of edible films made thereof. *Food Hydrocolloids, 29*(2), 280–289.

Medeiros, B. G., dos Souza, M. P. S., Pinheiro, A. C., Bourbon, A. I., Cerqueira, M. A., Vicente, A. A., et al. (2014). Physical characterisation of an alginate/lysozyme nano-laminate coating and its evaluation on 'Coalho'cheese shelf life. *Food and Bioprocess Technology, 7*(4), 1088–1098.

Mohammadi, R., Mortazavian, A. M., Khosrokhavar, R., & da Cruz, A. G. (2011). Probiotic ice cream: viability of probiotic bacteria and sensory properties. *Annals of Microbiology, 61*(3), 411–424.

Mokrzycki, W. S., & Tatol, M. (2011). Colour difference ΔE—a survey. *Machine graphics and vision, 20*(4), 383–411.

Niku-Paavola, M. L., Laitila, A., Mattila-Sandholm, T., & Haikara, A. (1999). New types of antimicrobial compounds produced by *Lactobacillus plantarum*. *Journal of Applied Microbiology, 86*(1), 29–35.

Özcelik, S., Kuley, E., & Özogul, F. (2016). Formation of lactic, acetic, succinic, propionic, formic and butyric acid by lactic acid bacteria. *LWT - Food Science and Technology, 73*, 536–542.

Ozdemir, M., & Floros, J. D. (2008). Optimization of edible whey protein films containing preservatives for water vapor permeability, water solubility and sensory characteristics. *Journal of Food Engineering, 86*(2), 215–224.

Pavli, F., Kovaiou, I., Apostolakopoulou, G., Kapetanakou, A., Skandamis, P., Nychas, G. E., et al. (2017). Alginate-based edible films delivering probiotic bacteria to sliced ham pretreated with high pressure processing. *International Journal of Molecular Sciences, 18*(9), 1867.

Perdones, A., Sánchez-González, L., Chiralt, A., & Vargas, M. (2012). Effect of chitosan–lemon essential oil coatings on storage-keeping quality of strawberry. *Postharvest Biology and Technology, 70*, 32–41.

Pereira, J. O., Soares, J., Sousa, S., Madureira, A. R., Gomes, A., & Pintado, M. (2016). Edible films as carrier for lactic acid bacteria. *LWT - Food Science and Technology, 73*, 543–550.

Pereira, O. J., Soares, J., Monteiro, M., Gomes, A., & Pintado, M. (2018). Impact of whey protein coating incorporated with *Bifidobacterium* and *Lactobacillus* on sliced ham properties. *Meat Science, 139*, 125–133.

Pérez, L. M., Piccirilli, G. N., Delorenzi, N. J., & Verdini, R. A. (2016). Effect of different combinations of glycerol and/or trehalose on

physical and structural properties of whey protein concentrate-based edible films. *Food Hydrocolloids, 56*, 352–359.

Pérez-Gago, M. B., & Krochta, J. M. (2001). Denaturation time and temperature effects on solubility, tensile properties, and oxygen permeability of whey protein edible films. *Journal of Food Science, 66*(5), 705–710.

Pérez-Gago, M. B., Nadaud, P., & Krochta, J. M. (1999). Water vapor permeability, solubility, and tensile properties of heat-denatured versus native whey protein films. *Journal of Food Science, 64*(6), 1034–1037.

Phan, T. D., Debeaufort, F., Luu, D., & Voilley, A. (2005). Functional properties of edible agar-based and starch-based films for food quality preservation. *Journal of Agricultural and Food Chemistry, 53*(4), 973–981.

Piermaria, J., Diosma, G., Aquino, C., Garrote, G., & Abraham, A. (2015). Edible kefiran films as vehicle for probiotic microorganisms. *Innovative Food Science and Emerging Technologies, 32*, 193–199.

Prema, P., Smila, D., Palavesam, A., & Immanuel, G. (2010). Production and characterization of an antifungal compound (3-phenyllactic acid) produced by *Lactobacillus plantarum* strain. *Food and Bioprocess Technology, 3*(3), 379–386.

Ramos, Ó. L., Fernandes, J. C., Silva, S. I., Pintado, M. E., & Malcata, F. X. (2012a). Edible films and coatings from whey proteins: a review on formulation, and on mechanical and bioactive properties. *Critical Reviews in Food Science and Nutrition, 52*(6), 533–552.

Ramos, Ó. L., Pereira, J. O., Silva, S. I., Fernandes, J. C., Franco, M. I., Lopes-da-Silva, J. A., Pintado, M. E., & Malcata, F. X. (2012b). Evaluation of antimicrobial edible coatings from a whey protein isolate base to improve the shelf life of cheese. *Journal of Dairy Science, 95*(11), 6282–6292.

Ramos, Ó. L., Pereira, R. N., Rodrigues, R. M., Teixeira, J. A., Vicente, A. A., & Malcata, F. X. (2016). Whey and whey powders: production and uses. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.), *Encyclopedia of food and health* (pp. 498–505). Oxford: Academic Press.

Ramos, Ó. L., Reinas, I., Silva, S. I., Fernandes, J. C., Cerqueira, M. A., Pereira, R. N., Vicente, A. A., Poças, M. F., Pintado, M. E., & Malcata, F. X. (2013). Effect of whey protein purity and glycerol content upon physical properties of edible films manufactured therefrom. *Food Hydrocolloids, 30*(1), 110–122.

Ramos, Ó. L., Silva, S. I., Soares, J. C., Fernandes, J. C., Poças, M. F., Pintado, M. E., & Malcata, F. X. (2012c). Features and performance of edible films, obtained from whey protein isolate formulated with antimicrobial compounds. *Food Research International, 45*(1), 351–361.

Reinoso, E., Mittal, G. S., & Lim, L.-T. (2008). Influence of whey protein composite coatings on plum (*Prunus domestica L.*) fruit quality. *Food and Bioprocess Technology, 1*(4), 314–325.

Reis, J. A., Paula, A. T., Casarotti, S. N., & Penna, A. L. B. (2012). Lactic acid bacteria antimicrobial compounds: Characteristics and applications. *Food Engineering Reviews, 4*(2), 124–140.

Rhee, S. J., Lee, J.-E., & Lee, C.-H. (2011). Importance of lactic acid bacteria in Asian fermented foods. *Microbial Cell Factories, 10*(1), S5.

Rizzello, C. G., Cassone, A., Coda, R., & Gobbetti, M. (2011). Antifungal activity of sourdough fermented wheat germ used as an ingredient for bread making. *Food Chemistry, 127*(3), 952–959.

Sánchez-González, L., Saavedra, J. I. Q., & Chiralt, A. (2013). Physical properties and antilisterial activity of bioactive edible films containing *Lactobacillus plantarum*. *Food Hydrocolloids, 33*(1), 92–98.

Sánchez-González, L., Saavedra, J. I. Q., & Chiralt, A. (2014). Antilisterial and physical properties of biopolymer films containing lactic acid bacteria. *Food Control, 35*(1), 200–206.

Schnurer, J., & Magnusson, J. (2005). Antifungal lactic acid bacteria as biopreservatives. *Trends in Food Science and Technology, 16*(1–3), 70–78.

Settier-Ramírez, L., López-Carballo, G., Gavara, R., & Hernández-Muñoz, P. (2019). Antilisterial properties of PVOH-based films embedded with *Lactococcus lactis* subsp. *lactis*. *Food Hydrocolloids, 87*, 214–220.

Soukoulis, C., Behboudi-Jobbehdar, S., Yonekura, L., Parmenter, C., & Fisk, I. (2014a). Impact of milk protein type on the viability and storage stability of microencapsulated *Lactobacillus acidophilus* NCIMB 701748 using spray drying. *Food and Bioprocess Technology, 7*(5), 1255–1268.

Soukoulis, C., Behboudi-Jobbehdar, S., Yonekura, L., Parmenter, C., & Fisk, I. D. (2014b). Stability of *Lactobacillus rhamnosus* GG in prebiotic edible films. *Food Chemistry, 159*, 302–308.

Soukoulis, C., Singh, P., Macnaughtan, W., Parmenter, C., & Fisk, I. D. (2016). Compositional and physicochemical factors governing the viability of *Lactobacillus rhamnosus* GG embedded in starch-protein based edible films. *Food Hydrocolloids, 52*, 876–887.

Soukoulis, C., Yonekura, L., Gan, H.-H., Behboudi-Jobbehdar, S., Parmenter, C., & Fisk, I. (2014c). Probiotic edible films as a new strategy for developing functional bakery products: The case of pan bread. *Food Hydrocolloids, 39*(100), 231–242.

Ström, K., Sjögren, J., Broberg, A., & Schnürer, J. (2002). *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides cyclo(l-Phe-l-pro) and cyclo(l-Phe-trans-4-OH-l-pro) and 3-phenyllactic acid. *Applied and Environmental Microbiology, 68*(9), 4322–4327.

Su, J.-F., Yuan, X.-Y., Huang, Z., Wang, X.-Y., Lu, X.-Z., Zhang, L.-D., & Wang, S. B. (2012). Physicochemical properties of soy protein isolate/carboxymethyl cellulose blend films crosslinked by Maillard reactions: Color, transparency and heat-sealing ability. *Materials Science and Engineering: C, 32*(1), 40–46.

Tapia, M. S., Rojas-Grau, M. A., Rodríguez, F. J., Ramírez, J., Carmona, A., & Martin-Belloso, O. (2007). Alginate- and gellan-based edible films for probiotic coatings on fresh-cut fruits. *Journal of Food Science, 72*(4), 190–196.

Tharanathan, R. N. (2003). Biodegradable films and composite coatings: past, present and future. *Trends in Food Science & Technology, 14*(3), 71–78.

Varsha, K. K., & Nampoothiri, K. M. (2016). Appraisal of lactic acid bacteria as protective cultures. *Food Control, 69*, 61–64.

Ye, J., Ma, D., Qin, W., & Liu, Y. (2018). Physical and antibacterial properties of sodium alginate - sodium carboxymethylcellulose films containing *Lactococcus lactis*. *Molecules, 23*(10), 2645.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.