Anti-E. coli cellulose-based materials

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A B S T R A C T

This work aimed to study Microcin J25(G12Y) (MccJ25(G12Y)) sorption to fibrous and microfibrillated cellulose (MFC) films for setting up anti-E. coli materials. The Freundlich model showed the best fit for both films, suggesting a physisorption process, and similar affinity between the peptide and both cellulose-based matrices, which in turn appeared to be equally heterogeneous. Migration studies showed complete release of MccJ25(G12Y) from the fibrous film after contact with water; meanwhile, the MFC film retained some activity after the experiment. Contact with fatty simulant had no impact on antimicrobial activity of fibrous film, whilst MFC showed a small decrease in its activity. However, MccJ25(G12Y) was not detected in the fatty simulant after active films contact. Fibrous and MFC films remained fully active during storage (3 weeks at 30°C). Finally, MccJ25(G12Y) activated fibrous film showed controlled release in conditions simulating critical steps in salmoni production. The results obtained suggest that fibrous and MFC films activated with MccJ25(G12Y) have a significant potential for their use as anti-E. coli materials in food settings.

1. Introduction

Food safety is receiving increasing attention worldwide, given the interdependence between food and health. Escherichia coli O157:H7 is the most important Shiga toxin-producing E. coli (STEC) serotype in relation to public health (WHO, 2018). Examples of food implicated in outbreaks of this pathogen include undercooked hamburgers, dried cured salami, unpasteurized fresh-pressed apple cider, yogurt, and cheese made from raw milk (WHO, 2018). The symptoms produced by infection with E. coli O157:H7 vary from mild to bloody diarrhea of which 10% of cases result in Hemolytic Uremic Syndrome (Farrokh et al., 2013).

In the endeavor to ensure food safety, biopreservation is a promising strategy that relies on the use of microorganisms and/or their metabolites, like bacteriocins (Vesković Moračanin, Dukić, & Memišić, 2014). The application of bacteriocins during food elaboration enables industries to meet consumer demands for food-safety, resulting at the same time in fresh and minimally processed foods (Mills, Stanton, Hill, & Ross, 2011). However, the spectrum of activity of bacteriocins is restricted to Gram-positive bacteria. Therefore, the development of a food biopreservative active against Gram-negative bacteria represents an attractive target.

The use of recombinant chymosin produced by E. coli K-12 in cheese manufacture (Flamm, 1991) has set a precedent for the application of well-characterized substances produced by E. coli in food settings. Microcin J25 (MccJ25) is a plasmid-encoded antimicrobial peptide produced by an enteric strain of E. coli. This peptide is active against several Gram-negative human pathogens comprising Salmonella spp., Shigella spp., and diarrheagenic E. coli, including E. coli O157:H7 (Sable, Pons, Gendron-Gaillard, & Cottencoue, 2000; Salomón & Farías, 1992). However, as MccJ25 resistance to proteolytic enzymes could affect the normal intestinal microbiota of the consumer when ingested, a derivative MccJ25(G12Y) sensitive to chymotrypsin was developed (Pavlíčková, Mukhopadhyay, Sineva, Ebright, & Severinov, 2008; Pomares et al., 2009). MccJ25(G12Y) was able to inhibit the growth of Salmonella enterica serovar Newport and E. coli O157:H7 in skim milk and egg.

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1 The interdisciplinarity involved in this work required equal collaborative efforts from Dr Blanco Massani and Dr Pomares.

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yolk. Moreover, MccJ25(G12Y) was inactivated by digestive enzymes both in vitro and in vivo (Pomares et al., 2009), suggesting that this peptide could be an effective additive to ensure food safety.

Antimicrobial biopreservatives have been successfully used for many years in the food industry. However, the characteristics and composition of food and the methodology applied during elaboration can influence the stability and activity of the biopreservative, making the addition of high concentrations necessary in order to ensure food safety (Veskić Moraćanin et al., 2014). In this regard, antimicrobial food packaging offers a great alternative, as this technology combines a reduced amount of antimicrobial with a controlled release from the packaging material to the food surface, where microbial contamination mainly occurs (Malhotra, Keshwani, & Kharkwal, 2015a).

Cellulose can be processed by different methods that lead to various structures and morphologies. This gives great versatility to cellulose-based materials for their application in passive and active food packaging (Jipa, Stoica-Guzun, & Stroescu, 2012; Malhotra, Keshwani, & Kharkwal, 2015b). Different bacteriocins were successfully incorporated into cellulose-based packaging films for potential applications in food preservation (Nguyen, Gidley, & Dykes, 2008; Scannell et al., 2000). However, hitherto, there are no reports on the use of bacteriocins and cellulose-based materials for the development of antimicrobial films that can fight E. coli. The aim of this work was to evaluate and compare the behavior of fibrous and microfibrilated cellulose (MFC) matrices as MccJ25(G12Y) carriers for the development of anti-E. coli films. The parameters for cellulose-based films activation with microcin were defined and sorption isotherms were built. Three isotherm models were fitted in non-linear form to describe the data generated from MccJ25(G12Y) sorption in fibrous and MFC matrices. Antimicrobial activity against E. coli AB1133, MccJ25(G12Y) migration from the active films using food simulants and antimicrobial stability over storage time were determined.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Escherichia coli SBG231(pG12Y) is the producer of MccJ25(G12Y). E. coli AB1133 is sensitive to the activity of MccJ25(G12Y) and was used as a surrogate for E. coli O157:H7. Both E. coli strains belong to the INSIBIO culture collection and were grown at 37°C in M9 medium (Sigma-Aldrich, St Louis, USA) supplemented with 1 mg/mL MgSO4, (Gicarelly, Santa Fe, Argentina), 1 mg/mL vitamin B1 (Sigma-Aldrich, St Louis, USA), 2 mg/mL tryptone (Sigma-Aldrich, St Louis, USA) and 2 mg/mL glucose (Sigma-Aldrich, St Louis, USA). All strains were stored at −70°C in 0.2 g/mL glycerol until use.

2.2. Cellulose-based films

Two cellulotic matrices were studied for their potential as microcin carriers, (i) a commercial synthetic casing Viscofan fibrous (Viscofan, 2016); (ii) an MFC film.

A detailed description of both materials can be found in the Supplementary material Section.

2.3. Microcin J25(G12Y) production and quantification in solution

MccJ25(G12Y) was obtained by purification of cell-free supernatant from E. coli SBG231(pG12Y), as detailed in Blond et al. (1999). Two techniques were used for microcin quantification, (i) UV absorbance: a standard curve of microcin concentration vs UV absorbance (275 nm) was built using a UV–visible spectrometer (UV-1800, SHIMADZU CORPORATION, Japan). (ii) The agar well diffusion method (Blanco Massani, Fernandez, Ariosti, Eisenberg, & Vignolo, 2008): aliquots (20 μL) of twofold dilutions of microcin solutions under study were spotted in wells performed on a semisolid M9 medium (7.5 mg/mL agar) inoculated with the sensitive indicator strain E. coli AB1133 (10⁶ CFU/mL). After incubation (18 h at 37°C), the plates were examined for zones of growth inhibition (halos) in the indicator lawn. MccJ25(G12Y) concentration was calculated considering that the highest dilution yielding a visible halo corresponds to 12.5 mg/L, which is the minimum inhibitory concentration (MIC) of MccJ25(G12Y), as determined in the present work.

2.4. Determination of MccJ25(G12Y) antimicrobial activity in cellulose films

Cellulose-based films treated with MccJ25(G12Y) (See section 2.5) were placed on semi-solid M9 agar plates inoculated with 10⁶ CFU/mL of indicator E. coli AB1133 strain. After overnight incubation at 37°C, the plates were examined for inhibition halos surrounding the materials. The halos were measured using ImageJ 471 software (Wayne Rasband, National Institutes of Health, USA), and expressed as relative inhibition area (RIA = film halos/film areas) (Blanco Massani et al., 2008).

2.5. Microcin sorption experiments

2.5.1. Determination of minimum contact time (MCT)

In order to determine the MCT needed to reach microcin sorption equilibrium in cellulose films, fragments of each sample (0.35 cm²) were immersed in an MccJ25(G12Y) solution (295 μL, 25 mg/L) for 5, 15, 30, 60 and 120 min at 30°C. After incubation, films were dried at room temperature and UV-sterilized; antimicrobial activity was determined as described in Section 2.4.

2.5.2. Sorption isotherm

To study the sorption isotherm, films were contacted with MccJ25(G12Y) solutions (from 6.25 to 800 mg/L) during the MCT at 30°C. Microcin concentration was quantified before and after the sorption process by UV absorbance (Section 2.3). The highest MccJ25(G12Y) concentration studied for film activation was chosen according to the decreased solubility of the peptide in water at concentrations around 1000 mg/L (data not shown).

MccJ25(G12Y) concentration sorbed to the films was calculated using the following equation:

\[ Q = \frac{(C_i - C_s)v}{g} \]  

(1)

where \( C_i \): MccJ25(G12Y) is the initial concentration, \( C_s \): the MccJ25(G12Y) concentration after the sorption process, and \( v \): the volume of microcin solution to which g (grams) of the film were contacted (Sarkar & Chattoraj, 1993).

After sorption experiments, the films were assessed for antimicrobial activity as described in Section 2.4. All film activation assays were carried out in two biological replicates with technical triplicates.

2.5.3. Sorption modelling

For reliable prediction of sorption parameters and quantitative comparison of sorbents behavior, isotherms obtained in Section 2.5.2 were fitted to three isotherm models in non-linear form: Langmuir, Freundlich, and Dubinin–Radushkevich.

The parameters given by the different models were evaluated by non-linear regression using Origin Pro 8 (V8.0724) software. The goodness of all fits to experimental data was followed by the correlation coefficient (\( R^2 \)), the residual sum square error (SSE) and the chi-square test (\( \chi^2 \)). The best-fit model should have the least SSE and \( \chi^2 \), as well as the highest \( R^2 \) (Vijayaraghavan, Padmesh, Palanivelu, & Velan, 2006).
2.6. McCJ25(G12Y) antimicrobial stability and migration from cellulose materials to food simulators

The following assays were carried out in films treated with a 200 mg/L McCJ25(G12Y) solution. This concentration was chosen to ensure a microcin release higher than the peptide MIC.

Antimicrobial stability of the films was evaluated by activity determination (Section 2.4) at time 0, and after 1, 2 and 3 weeks of storage at 30°C.

To study McCJ25(G12Y) migration, the active cellulose-based films were incubated separately in 295 μL of water and sunflower oil at 40°C in order to assemble aqueous and fatty food (MERCOSUR/GMC/RES. N°32/10, 2010). After 10 days of contact, film samples were removed and evaluated for residual antimicrobial activity as described in Section 2.4. A dry active film stored at 40°C for 10 days was used as microcin positive control. Antimicrobial activity of water and sunflower oil after film removal was determined by the agar well diffusion assay (Section 2.3).

Additionally, considering that fibrous matrix is used as a casing for sausage fermentation (Viscofan, 2016), the release of microcin from active fibrous film was studied in contact with components used for salami production. The fibrous film to stuff proportion was calculated based on the caliper of fibrous casing and the bater composition for salami (Ross & Shadbolt, 2001). Accordingly, the active fibrous film (0.35 cm²) was contacted separately with 0.6 g of bacon, 0.6 g of meat and 205 μL of salt water (containing 5.53 mg NaCl, 1.25 mg phosphates, 0.2 mg erythorbate, 0.08 mg NaNO₂ and 0.04 mg NaNO₃). Incubation was performed for 24 h at 24°C and then for 48 h at 18°C (Ross & Shadbolt, 2001). After contact, residual activity of the film and antimicrobial activity of the saline solution, bacon and meat were evaluated in semisolid agar (Sections 2.3 and 2.4).

2.7. Statistical analysis

Minitab Statistic release 12 (Pennsylvania, USA) was used. Experimental data were subjected to an analysis of variance (ANOVA) and Tukey’s test was applied with a significance level of 95%.

3. Results

3.1. Kinetics and sorption equilibrium of McCJ25(G12Y) on cellulose-based films

The minimum contact time (MCT) necessary to obtain cellulose-based films activated with McCJ25 was studied. Table 1 shows the relative inhibition areas (RIA) obtained after the fibrous and MFC films were contacted with 25 mg/L of McCJ25(G12Y) for 5, 15, 30, 60 or 120 min at 30°C. For each set of films, the RIAs showed no significant differences at the analyzed times, defining an MCT of 5 min (Table 1). Additionally, it was observed that the RIA values were in the same order for fibrous and MFC films.

Table 1 Antimicrobial activity of the fibrous and MFC films after immersion in McCJ25(G12Y) for different time periods.

<table>
<thead>
<tr>
<th>Contact time (min)</th>
<th>Relative inhibition areas</th>
<th>MFC film</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fibrous film</td>
<td>MFC film</td>
</tr>
<tr>
<td>5</td>
<td>3.67 ± 1.08</td>
<td>2.85 ± 0.33</td>
</tr>
<tr>
<td>15</td>
<td>3.74 ± 0.69</td>
<td>3.05 ± 0.45</td>
</tr>
<tr>
<td>30</td>
<td>3.77 ± 1.27</td>
<td>3.41 ± 0.57</td>
</tr>
<tr>
<td>60</td>
<td>3.24 ± 1.06</td>
<td>3.25 ± 0.55</td>
</tr>
<tr>
<td>120</td>
<td>3.51 ± 0.65</td>
<td>3.15 ± 0.27</td>
</tr>
</tbody>
</table>

Values represent means ± standard deviation (SD) of six-fold replicates.

3.2. Technological properties of cellulose-based films activated with McCJ25(G12Y)

3.2.1. McCJ25(G12Y) antimicrobial stability and migration from films

The useful life of active fibrous and MFC films stored at 30°C was studied (Table 3). The results obtained presented high variability, which was intimately related to the E. coli AB1133 strain variable sensitivity. Therefore, to facilitate results interpretation, activity respect to the control was reported (RIA of sample/RIA of the positive control evaluated on the same day as the experiment) (Table 3). For both films, inhibition areas relative to the positive control showed no antimicrobial reduction during the storage period, indicating that cellulose-based matrices remained fully active when stored for 3 weeks at 30°C.

In order to investigate which type of food could be efficiently protected using fibrous or MFC active films, water and sunflower oil, representing aqueous and fatty food simulants, were used to determine microcin migration from these matrices. As shown in Table 3, after water contact at 40°C, 26.50 ± 0.40 mg/mL of McCJ25(G12Y) were released from fibrous film, while no residual activity was detected in the fibrous matrix. In contrast, 20% of residual activity was found in
Table 2
Isotherm models and parameters obtained for MccJ25(G12Y) sorption in fibrous and MFC films.

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameters</th>
<th>Fibrous</th>
<th>MFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Langmuir isotherm</td>
<td>$Q_{max}$</td>
<td>75.51 ± 39.30$^a$</td>
<td>17.66 ± 9.00$^b$</td>
</tr>
<tr>
<td></td>
<td>$b_L$</td>
<td>0.0002 ± 0.0001$^a$</td>
<td>0.0005 ± 0.0003$^a$</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.9834</td>
<td>0.8764</td>
</tr>
<tr>
<td></td>
<td>SSE</td>
<td>3.59</td>
<td>11.96</td>
</tr>
<tr>
<td></td>
<td>$X^2$</td>
<td>0.2247</td>
<td>0.2992</td>
</tr>
<tr>
<td>Freundlich isotherm</td>
<td>$K_F$</td>
<td>0.028 ± 0.007$^a$</td>
<td>0.020 ± 0.009$^a$</td>
</tr>
<tr>
<td></td>
<td>$n_F$</td>
<td>1.10 ± 0.05$^a$</td>
<td>1.21 ± 0.11$^a$</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.9841</td>
<td>0.8783</td>
</tr>
<tr>
<td></td>
<td>SSE</td>
<td>3.44</td>
<td>11.78</td>
</tr>
<tr>
<td></td>
<td>$X^2$</td>
<td>0.2150</td>
<td>0.2945</td>
</tr>
<tr>
<td>Dubinin-Radushkevich isotherm</td>
<td>$Q_d$</td>
<td>10.66 ± 0.72$^a$</td>
<td>4.81 ± 0.39$^b$</td>
</tr>
<tr>
<td></td>
<td>$b_d$</td>
<td>0.0067 ± 0.0012$^a$</td>
<td>0.0088 ± 0.0019$^a$</td>
</tr>
<tr>
<td></td>
<td>$E$</td>
<td>8.64 ± 0.77$^a$</td>
<td>7.53 ± 0.81$^a$</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.9075</td>
<td>0.7791</td>
</tr>
<tr>
<td></td>
<td>SSE</td>
<td>24.52</td>
<td>18.96</td>
</tr>
<tr>
<td></td>
<td>$X^2$</td>
<td>1.2381</td>
<td>0.5748</td>
</tr>
</tbody>
</table>

$^a$, $^b$ Values with different letters in the same line present significant differences (P < 0.05).
Values represent means ± standard error (SE) of six-fold replicates.

MFC after water contact (Table 3) and the concentration of MccJ25(G12Y) released to the water was 21.88 ± 5.79 mg/L.

After contact with the fatty simulant, the antimicrobial activity of the fibrous film showed no significant differences respect to the MccJ25(G12Y) positive control film (P = 0.876). However, for the activated MFC counterpart a slight decrease in antimicrobial activity was observed (P = 0.003) (Table 3).

3.2.2. MccJ25(G12Y) release from fibrous film upon contact with salami ingredients

To ascertain MccJ25(G12Y) migration and/or inactivation in conditions resembling fermentation and drying stages in salami, microcin activated fibrous film was incubated for 24 h at 24°C and then for 48 h at 18°C in contact with salami ingredients (Table 4). After 24 h at 24°C, a decrease in film RIA from 6.54 ± 0.62 to 3.55 ± 0.34 and 2.93 ± 0.20 was observed upon contact with meat and salt water, respectively (Table 4). This decrease in film activity was accompanied by a release of microcin to the corresponding food matrices, since meat RIA increased from 0 to 2.91 ± 0.20, and 37.50 ± 12.50 mg/L of microcin were found in salt water (Table 4). Upon bacon contact (24 h at 24°C), activity reduction in the fibrous film could not be distinguished due to the variability of the experiment; however, antimicrobial activity was found in the foodstuff (bacon RIA of 2.05 ± 0.40) (Table 4). When conditions were changed to resemble the drying process during salami production (48 h at 18°C) (Ross & Shadbolt, 2001), microcin activity in bacon increased up to an RIA of 3.21 ± 0.71, with the corresponding decrease in the fibrous film from 5.88 ± 1.56 to 3.63 ± 0.85. Meanwhile, after meat contact, activity was maintained for both the film and the food matrix (Table 4). In contrast, after 48 h at 18°C, a reduction in microcin activity for the fibrous film after water salt contact was observed (film RIA from 2.93 ± 0.20 to 1.98 ± 0.13), with no changes in the concentration of microcin in salt water (37.50 ± 17.70 mg/L) (Table 4). Microcin activity was not affected by cultures commonly used as starters for sausage fermentation (data not shown).

4. Discussion

Considering the potential that MccJ25(G12Y) has for fighting pathogenic strains of E. coli (Pomares et al., 2009), the development of active anti-E. coli cellulose-based films was proposed in this work. The cellulose-based matrices used in this study (fibrous and MFC) presented different morphology (details can be found in Supplementary material). Defining the minimum time required to obtain active materials (MCT) is the first step to set up antimicrobial materials by sorption (Massani, Vigolo, Eisenberg, & Morando, 2013). Our results indicated that

Table 3
Antimicrobial activity of the films over storage time; residual activity and concentration in food simulants after migration experiments. For detailed information see Section 2.6.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fibrous film</th>
<th>MFC film</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity with respect to the control$^a$</td>
<td>Concentration in simulant (mg/L)</td>
</tr>
<tr>
<td>Positive control</td>
<td>1.00 ± 0.23$^a$</td>
<td>na</td>
</tr>
<tr>
<td>Stored 1 week</td>
<td>1.28 ± 0.36$^a$</td>
<td>na</td>
</tr>
<tr>
<td>Stored 2 weeks</td>
<td>1.23 ± 0.47$^a$</td>
<td>na</td>
</tr>
<tr>
<td>Stored 3 weeks</td>
<td>1.47 ± 0.85$^a$</td>
<td>na</td>
</tr>
<tr>
<td>Positive control for migration</td>
<td>1.00 ± 0.35$^a$</td>
<td>na</td>
</tr>
<tr>
<td>Water contacted</td>
<td>0.60 ± 0.00$^b$</td>
<td>26.50 ± 0.40</td>
</tr>
<tr>
<td>Sunflower oil contacted</td>
<td>0.98 ± 0.33$^a$</td>
<td>nd</td>
</tr>
</tbody>
</table>

Values represent means ± SD of six-fold replicates.
$^a$, $^b$ Different letters in the same column represent significant differences (P < 0.05).
nd, not detected.
na, not applicable.
$^a$ Activity respect to the control (RIA/RIA of the positive control).
sorption equilibrium was reached after 5 min of contact with MccJ25(G12Y) for both cellulose-based films. The factors affecting peptide adsorption on surfaces are numerous and entailed peptide characteristics, surface properties, the surrounding medium, and the interactions between peptide and surface (Karam, Jama, Dhubler, & Chihih, 2013). Nisin is the antimicrobial peptide most extensively studied and exploited for setting up antimicrobial surfaces. Therefore, it can serve as a model to encourage the emergence of new bacteriocins and new potential bio-preservatives (Karam et al., 2013). The conditions found in the literature for nisin adsorption to cellulose-based polymers are diverse. Periods of incubation range from 6 to 8 h at 4 °C (Nguyen et al., 2008; Scannell et al., 2000), whilst 12 h were required at 25 °C (Perez Guerra, Lopez Macias, Torrado Agranar, & Castro, 2005). As addressed for sorption in other systems, equilibrium time can be controlled by the adsorbate molecular size, i.e., the larger the size, the longer the diffusion time (Muñoz, Jarrah, Zubair, & Alagha, 2017). The lower contact time observed here for MccJ25(G12Y) sorption on cellulose matrices (5 min in contrast to several hours) is in line with its lower molecular size (2.1 kDa) compared to nisin (3.5 kDa).

Equilibrium isotherms were modelled in order to understand the sorption behavior of microcin in fibrous and MFC films. From the results obtained, the following can be inferred: (i) similar MccJ25(G12Y) affinity was obtained for fibrous film and MFC (bL values: 0.0002 ± 0.0001 and 0.0005 ± 0.0003, respectively, Table 2); (ii) fibrous film would present the same heterogeneity as MFC (1/nL, 0.9090 and 0.8264, respectively); (iii) mean free energy change of adsorption (8.64 ± 0.77 and 7.53 ± 0.81 kJ/mol, respectively, for fibrous and MFC matrices) indicated moderate sorption intensity (physiosorption) between MccJ25(G12Y) and both cellulose-based materials (more details on the discussion of the models can be found under Supplementary material).

Bacteriocin stability in active materials ensures full protection during food product shelf-life. It has been reported that cellulose-based films activated with nisin lost their activity after one week of storage at 4 °C (Scannell et al., 2000). In contrast, we showed that antimicrobial activity of MccJ25(G12Y) in films remained intact for at least 3 weeks of storage at 30 °C. This can be attributed to the structural features of the peptide (Pomares et al., 2009; Vincent & Morero, 2009).

The goal of active packaging is to reduce bacterial growth. In this context, antimicrobial controlled release is advantageous since it allows for longer and more effective antimicrobial action in food. A total release of the peptide from the fibrous film after water contact was observed, while a small percentage of the sorbed microcin was retained in the MFC matrix. This difference in microcin release behavior from both cellulose-based matrices could be an indirect evidence that, besides adsorption of MccJ25(G12Y) on the MFC surface, peptide partition into the polymer matrix could be taking place. Lysozyme controlled and limited release from MFC films was linked to MFCs nano-sized features (Cozzolino et al., 2013). Likewise, it could be hypothesized that unreleased MccJ25(G12Y) may be trapped into an MFC nanoporous network. From a technological point of view, the behavior found for our anti-E. coli MFC film could be promising to achieve antimicrobial controlled release to food systems. Moreover, MccJ25(G12Y) was released at least at the MIC when active cellulose materials made contact with an aqueous simulant (Table 3). This suggests that cellulose-based anti-E. coli materials, paired with good manufacturing practices, may have the potential to contribute as an additional hurdle to increase the safety of aqueous food such as meat cuts, hamburgers and ground beef, avoiding possible risks related to E. coli O157:H7 outbreaks.

MccJ25(G12Y) activated materials can also be proposed for their use in cheese and fermented meat products, which were involved in E. coli O157:H7 outbreaks (Farrokh et al., 2013; Ross & Shadbolt, 2001). Considering the hydrophobic nature of microcin (Vincent & Morero, 2009), it might be expected that the peptide would migrate to fatty simulant. However, no antimicrobial activity was found in sunflower oil after contact with the active cellulose-based films. While water is a strong solvent for cellulose polymer matrices, hydrophobic media has an only partial affinity and may trigger the release of the active compound to a lesser extent (Cozzolino et al., 2013). The results obtained here may indicate that MccJ25(G12Y) is migrating at non-detectable concentrations from cellulose-based films to the fatty simulant.

Salami is a sausage, whose fermentation and drying stages produce physicochemical changes that condition the safety, stability and sensory characteristics of the final product (Ross & Shadbolt, 2001). The fermentation process can last between 24 and 72 h and seeks to avoid the growth of pathogens that can contaminate raw meat. The transition from growth to non-growth of E. coli would be expected to occur at some point during the fermentation process (Ross & Shadbolt, 2001). Therefore, fermentation is a critical step for salami safety. Migration results from fibrous activated with MccJ25(G12Y) showed the partial release of the antimicrobial in all components tested, ensuring a level higher than the MIC during and after the critical hours that guarantee salami safety (Ross & Shadbolt, 2001). However, the reduction of microcin activity found for fibrous upon salt water contact (48 h at 18 °C) may indicate that this condition affects the antimicrobial stability of the peptide. Nevertheless, the active concentration released to salt water was approximately threefold higher than the MIC against the surrogate for E. coli O157:H7 (Table 4). Altogether, this demonstrates that microcin was released at conditions mimicking the salami fermentation process. Thus, the fibrous film activated with MccJ25(G12Y) seems to be a promising antimicrobial material to warrant the final reduction of E. coli O157:H7 in fermented products.

5. Conclusion

Anti-E. coli fibrous and MFC films were obtained by contact (5 min) with MccJ25(G12Y) solution. No saturation point was observed for either cellulose matrix, suggesting that a high spectrum of MccJ25(G12Y) concentrations can be included into these films. The Freundlich model best fitted experimental data. MccJ25(G12Y) was
physiosorbed to fibrous and MFC films with a similar affinity. Anti-E. coli cellulose materials retained their activity up to 3 weeks under storage at 30 °C. Active films released MccJ25(G12Y) after 10 days in contact with water. MccJ25(G12Y) migration from cellulose-based matrices after contact with sunflower oil was not detected. Migration studies performed in contact with salami ingredients showed promising results, as the active fibrous film was able to release effective concentrations of MccJ25(G12Y) in meat, bacon and salt water during and after the critical hours that ensure salami safety. From the sorption characteristics and technological properties studied in the present work, it can be concluded that fibrous and MFC films activated with MccJ25(G12Y) have a significant potential for their use as anti-E. coli materials in food products.

The projection of this study is the development of anti-E. coli materials potentially applicable to the meat and dairy industries. Part of the future studies will involve the addition of surfactants to the cellulose-based materials in order to increase micronic migration to fat simulants, together with the effectiveness assessment in meat and dairy products.

Conflicts of interest

The authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2019.02.084.

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