Use of alginate-based films as a carrier for bacteriophages: antimicrobial assessment against *Pseudomonas fluorescens*

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Introduction

The use of bacteriophages (phages) to improve safety and quality in food products is growing exponentially, increasing the interest by the scientific and industrial community in knowing their activity, action behaviour and bioavailability in food systems (acting as a way to control food borne pathogens). These possibilities can be converted into real applications exploring the functions and properties of bio-based structures and using them for bacteriophage protection, since when applied in the free form bacteriophages lose viability (e.g. in gastrointestinal conditions). Understand which incorporation structures are the most appropriate, their properties and behaviour using alternative food-grade materials (e.g. polysaccharides) to incorporate phages should be addressed aiming at innovative functions with enhanced functionality and a safe application of bacteriophages in food products.

Materials and Methods

Film-forming solutions were prepared by dissolution of 1% (w/v) of alginate in water under agitation during 3 h. Glycerol (0.5%) was added and the solutions were homogenized under agitation (overnight). Then bacteriophage ΦIBB-PF7A (7.7%) with 5E3 plaque forming units (PFU) per millilitre (mL) were added and stirred (approximately 30 min). Solutions were cast into polystyrene Petri dishes of 45 mm, and dried at 30 ˚C during 48 h. Calcium chloride ($CaCl_2$) solution 1% (m/v) was cast in the formed alginate films and being the excess removed (**Figure 1**). Films were dried at ambient temperature and then conditioned at 53% of RH and 20 ˚C before moisture and solubility measurements (Galus & Kadzińska, 2016). Antimicrobial activity of films with phage was tested using chicken meat. Alginate films were placed in sample cups bottom and chicken meat (2 g of meat infected with 3.15E7 CFU of *P. fluorescens*) was placed upon the film at 4 ˚C for 24 h. Chicken samples PFU and CFU and films PFU were measured along the time using small drop plaque assay method (**Figure 2**).

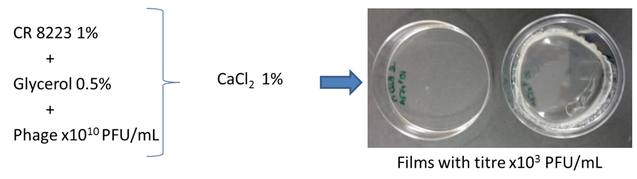

Figure 1. Phage entrapment in alginate films

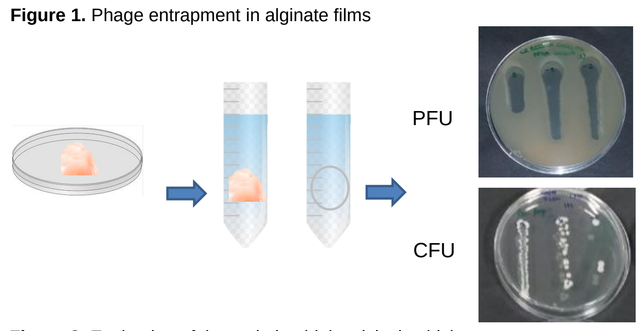

Figure 2. Evaluation of the antimicrobial activity in chicken meat

Results

Different types of alginate were tested with different mannuronic/guluronic ratios. After film formation it was possible to select the best one regarding the objective of using them as a substitute of chicken meat cuvettes absorbents: alginate CR 8223 due to better texture and appearance was selected. Different concentrations (0-1.5%) of Alginate CR 8223 and of cross linker ($CaCl_2$) (0-1.5%) were tested (**Figure 3**).

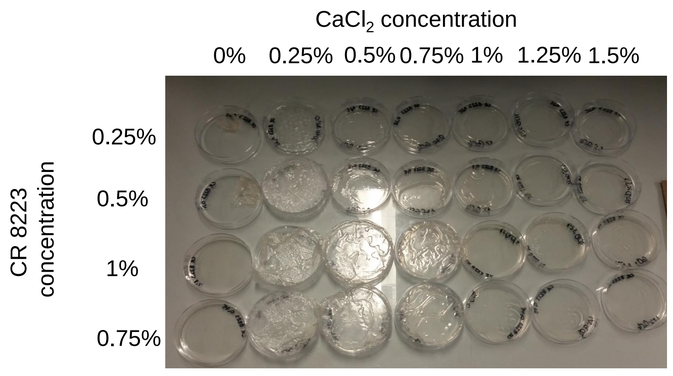

Figure 3. Films with different concentrations of Alginate CR 8223 (0%, 0.25%, 0.75%, 1%) and different concentrations of $CaCl_2$

Moisture and solubility were determined for Alginate CR 8223 1% with different concentrations of $CaCl_2$ (0%, 1%, 1.25%, 1.5%). The values are presented Figure 4 and 5.

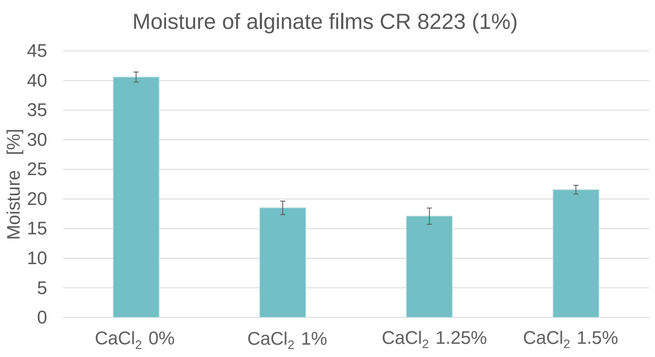

Figure 4. Moisture content of alginate CR 8223 (1%) with different $CaCl_2$ concentrations (0%,1%,1.25%,1.5%) films

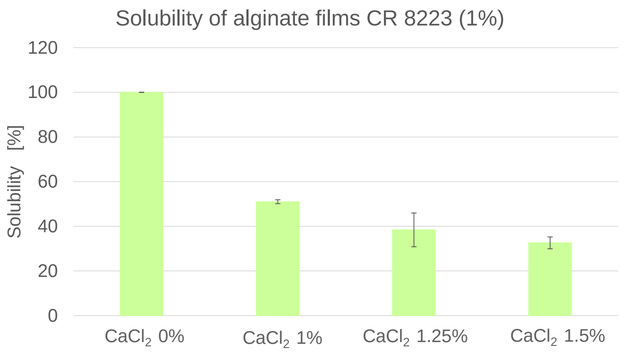

Figure 5. Solubility of alginate CR 8223 (1%) with different $CaCl_2$ concentrations (0%,1%,1.25%,1.5%) films

Moisture and solubility was decreased with the use of $CaCl_2$ as cross-linker. Contact angle was measured. Results are presented in **Table 1**. Crosslinked films present lower hydrophilicity.

Table 1. Contact angle of Alginate CR 8223 1% with different $CaCl_2$ concentrations (0%,1%,1.25%,1.5%) films

	Contact angle [˚]
CR 8223 1% $CaCl_2$ 0%	37.35±2.24
CR 8223 1% $CaCl_2$ 1%	30.76±3.35
CR 8223 1% $CaCl_2$ 1.25%	33.26±2.54
CR 8223 1% $CaCl_2$ 1.5%	45.34±2.59

After the production of alginate films with bacteriophage ΦIBB-PF7A, antimicrobial activity was evaluated in chicken meat (**Figure 6**). Overall results are presented in **Table 2**.

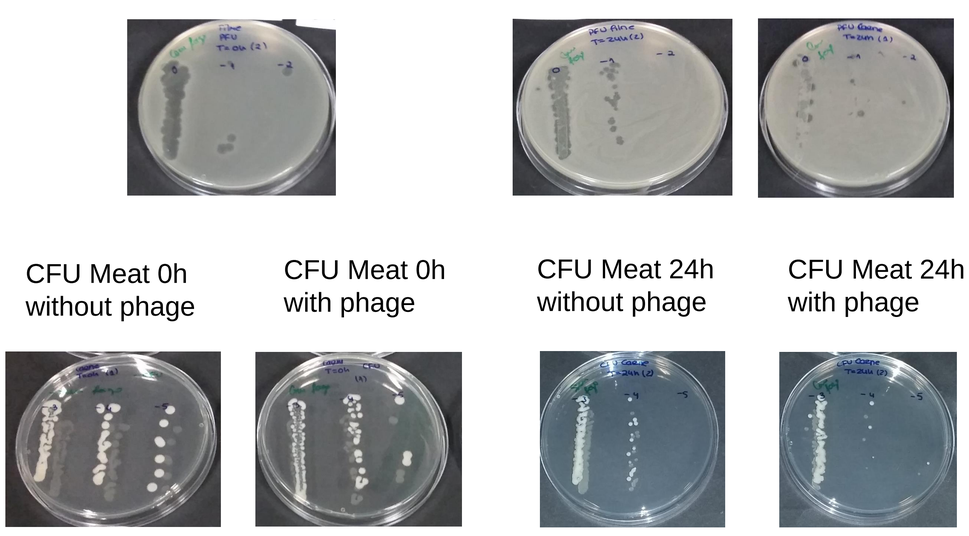

Figure 6. PFU of films at 0 and 24 h, PFU of meat at 24h and CFU of *P. fluorescens* in meat with and without phage at 0 and 24h

It is possible to notice that after 24h the phage is present in the meat, that shows a migration occurred from the film to the meat. This results indicate that the film allows the release of the phage into the meat.

Table 2. Results of antimicrobial activity in chicken meat

	0h	24h
CFU/mL (with phage)	3.15 x 10^7	2.5 x 10^6
CFU/mL (without phage)	2.10 x 10^7	1.30 x 10^7
PFU/mL (in film)	5.00 x 10^3	1.95 x 10^4
PFU/mL (in meat)	0	2.25 x 10^3

Looking at the results it is possible to observe a decrease of *P. fluorescens* in the meat after 24 h and ensure the antimicrobial activity of the produced films.

Conclusions

The best alginate film was obtained using an alginate with 0.65/0.35 of M/G ratio (CR 8223) and a concentration of 1% (m/v). Regarding the cross-linker $CaCl_2$ the best concentration was 1% (m/v). The optimized time and method for crosslinking were 5 minutes and the casting method, respectively. The chosen alginate based-film was able to maintain ΦIBB-PF7A phage activity showing a good titre value. Results showed that bacteriophage ΦIBB-PF7A can be incorporated in alginate-based films, maintain their activity and being released into chicken meat leading to a decrease of *P. fluorescens* contamination. Consequently, there is a clear industrial potential of using alginate-based films with bacteriophages as a substitute of common poultry cuvettes absorbents pads.

References

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