Electrospun nanofibres as a novel encapsulation vehicle for Felix O1 bacteriophage for new food packaging applications

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Abstract

Background & Objectives: Food contamination is one of the main issues in the food industry, and food packaging has been driven towards novel technologies to reduce bacterial contaminations. One of the possibilities is the development of antibacterial packaging by the encapsulation of bacteriophages, namely Felix O1, can provide protection against microbial contaminations in the food chain. This work aimed the encapsulation of Felix O1 into electrospun nanofibres to be used in biodegradable packaging as a controlling agent of Salmonella Enteritidis in foods.

Methods and Results: After the optimization of different electrospinning conditions (voltage, flow rate, polymer type, polymer concentration and type of solvent), polypyrrole alcohol (PVPH) with Felix O1 bacteriophage solution was electrospun on polyhydroxybutyrate/polyhydroxyvalerate film forming a layer composed by sub-micro nanofibres. The optimized conditions were: SM buffer solution of PVPH at 14% (w/v) at a flow rate 0.3 mL/h and applied voltage of 25 kV.

Conclusion: After the formation of nanofibres with a size around 100 nm (observed through Scanning Electron Microscopy), release tests in SM buffer for 1 h revealed a high bacteriophage viability (10^6-10^8), but still, there was a decrease of two log in the expected phase titre. DSC and TGA results revealed differences between the films and the films with nanofibres, showing the influence of the nanofibres in the system namely in the thermal behaviour. Results show that this new packaging system is promising for the development of active packaging using bacteriophages.

Significance and impact of the study: The encapsulation of bacteriophages through electrospinning shows high potential as a new feature in food packaging, as a possible solution for bacterial contamination in foods.

Introduction

Food industry has on the production chain several stages that are continually changed in order to improve quality and safety of food products and develop products as a way to improve wellness for consumers, as a consequence of the increasing know-how in the food technologies. One of the major problems consists in microbial contamination leading to food spoilage and foodborne illnesses.6-8 The main objective of this work is the development and characterization of bio-based structures at micro- and nano scale for encapsulation of bacteriophages and the establishment of a relationship between their properties and activity.

Methods

Nanofibres production

PVPH 14% in SM Buffer Gelatin 2 %, E. coli bacteria

Addition of 12.850 rpm Room temperature

Electrospinning 30 kV, 0.1 mm

Conditioning (4°C) for release tests and Conditioning (50% RH, 120°C) – other tests

Results

Nanofibres optimization

Selection of matrix deposition

Water Vapour permeability

Table 1. Values of TA-Disc measurement temperature \(T_{\text{sec}}\) and \(T_{\text{disc}}\)

<table>
<thead>
<tr>
<th>Sample</th>
<th>(T_{\text{sec}})</th>
<th>(T_{\text{disc}})</th>
<th>(\Delta T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVPH</td>
<td>283.4</td>
<td>141.5</td>
<td>135.9</td>
</tr>
<tr>
<td>PHBV nanofibre</td>
<td>274.0</td>
<td>209.5</td>
<td>164.5</td>
</tr>
</tbody>
</table>


d) Release tests in SM buffer for 1 h revealed a high bacteriophage viability (10^8-10^10), but still, there was a decrease of two log in phase titre (Table 3). 14% of PVPH diluted in SM Buffer and 0.01% Gelatin was the selected formulation and 0.3 mL/h and 25 kV were the conditions used in the electrospinning process, regarding fibres homogeneity and processability.

Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA)

- PVPH + nanofibre presented lower melting temperatures compared to PHBV. Both samples had a step melting behaviour probably due to the existence of crystals that recrystallize after melting forming more crystals that melt at higher temperatures.
- PVPH films presented 283.4 °C as degradation temperature while for PHBV films with nanofibre (PVPH 14%-SM buffer, Felix O1) the highest degradation was around 274 °C but a second degradation temperature appeared at 290.5 °C.

Table 2. Values of Tg-Disc measurement temperature \(T_{\text{sec}}\) and \(T_{\text{disc}}\)

<table>
<thead>
<tr>
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• PVPH and PHBV films allow to produce improved PVPH films comparing with PLA films.

Conclusions

PVPH nanofibres deposited in PHBV films allow the production of homogeneous fibres with a diameter around 100 nm. Bacteriophages are able to be incorporated into PHBV-nanofibres through electrospinning and maintain a high activity (10^6-10^9 PFU).

Nanofibres presence results in higher WVP due to PVPH, also TGA and DSC results showed nanofibre influence in the thermal behaviour.

Further tests of moisture, suitability, mechanical properties and retention and release tests in liquid and solid simulants are important to understand the fibre performance.

These studies will promote the use of bacteriophages in food applications to ensure food safety and an increasing knowledge in now active systems where the information is still scarce.