Controlled Release of Nisin from Biopolymer Films

Jino Chacko¹, Minal Lalpuria², John Floros², Ramaswamy Anantheswaran²

¹General Mills Inc., Minneapolis, USA (jino.chacko@generalmills.com)
²Department of Food Science, The Pennsylvania State University, University Park, USA
(mpl161@psu.edu, jdf10@psu.edu, rca3@psu.edu)

ABSTRACT

Nisin is a bacteriocin approved as a food preservative. In food systems, nisin loses its antimicrobial activity over time because it binds to proteins, fats, etc. This problem can be alleviated by controlling the overall release rate of nisin into foods. This research was undertaken to evaluate various biopolymer-based films for use in controlled-release of nisin into aqueous food systems. The objectives of this study were to investigate various biopolymers – locust bean gum (LBG), xanthan gum (XG), carrageenans (CA), alginates (AL), hydroxypropylmethyl cellulose (HPMC) and corn zein (CZ) – for nisin release, and to study their antimicrobial activity by the agar diffusion method. XG and LBG films quickly dissolved on the agar surface without producing a distinguishable inhibition zone. Similarly, kappa and iota CG films did not produce any inhibition zone. Blended films made with kappa CA and HPMC exhibited measureable nisin release and the inhibition zone increased with increasing HPMC concentration in the film. AL and CZ film formed clear inhibition zones. For AL films, the inhibition zone decreased with increasing crosslinking time and % CaCl²; while the zone size increased with higher % guluronic acid. The amount of nisin released from CZ films was quantified using a high performance liquid chromatographic (HPLC) technique, and a Weibull model was fitted to the data. Nisin release decreased as the concentration of corn zein in the film matrix increased. Films made with HPMC, AL and CZ showed promise for developing controlled release applications with nisin in aqueous food systems.

Keywords: controlled release; nisin; corn zein; alginate

INTRODUCTION

Nisin is the only bacteriocin that can be used as a natural food preservative [1, 2] and has been approved as “generally recognized as safe” (GRAS) substance by both the Food & Drug Administration (FDA) and the World Health Organization (WHO). Nisin is commercially available as Nisaplin®. Pure nisin has an activity of 40 x 10⁶ IU/mg, while Nisaplin® contains 2.5% nisin and has an activity of 10⁶ IU/g (Danisco USA Inc., New Century, KS). It has been observed that in some food systems, especially meat, nisin loses its activity over time. Biopolymers, such as locust bean gum (LBG), xanthan gum (XG), carrageenans (CA) (kappa, iota and lambda), hydroxypropylmethyl cellulose (HPMC), alginates (AL) and corn zein (CZ) were evaluated for controlled release of nisin in an aqueous system. The objectives of this research were to:

1) Evaluate different biopolymer-based films for controlled release of nisin;
2) Investigate the effect of crosslinking time, % CaCl² and % guluronic acid on the release of nisin from alginate films;
3) Determine the effect of CZ concentration (4, 6, 8 and 10% w/v) on the release kinetics of nisin into an aqueous system and to model the release of nisin through these films.

MATERIALS & METHODS

Film preparation

1% (w/v) LBG and XG films were prepared by dissolving the biopolymers in deionized water. CA 2% (w/v) films (kappa, iota and lambda) were developed according to the procedure outlined in [3]. Since CZ is not miscible in water, cast CZ films were prepared by dissolving varying concentrations of CZ in 95% ethyl alcohol [4-6]. Based on preliminary experiments, CZ films with concentrations 4, 6, 8 and 10% (w/v), were used for this research. Alginate is an anionic polymer composed of mannuronic acid (M) and guluronic acid (G), linked together by (1, 4) glycosidic bonds to form linear molecules. 1% (w/v) AL films were prepared...
by dissolving sodium alginate in deionized water at 70°C. The film solution was then cooled to room temperature and 20% (v/w) glycerol/sodium alginate and Nisaplin® (Danisco, USA) was added. The films were cast by pouring 40 ml of film solution in 17.5cm x 7cm teflon plates and dried in a controlled environment chamber at 25°C and 50% RH.

Antimicrobial activity of nisin containing films
An agar diffusion method was used to verify the antimicrobial activity of the films, according to the procedure outlined in [7]. The media consisted of nutrient broth, 0.75% Bacto™ agar and 1% Tween 20. The media was inoculated with 1% (v/v) Micrococcus luteus, an indicator organism sensitive to nisin. Seven mm discs were cut from the films and placed on agar plates seeded with M. luteus, followed by incubation at 37°C. The inhibition zones formed were observed after 24-48 hrs of incubation.

Nisin quantification by HPLC method
A C-18 reverse-phase column was used for quantifying nisin [8, 9]. Solutions having nisin activity 0 to 15000 IU/ml were prepared, by serial dilutions of a stock solution of Nisaplin®. The nisin peak area in the chromatogram was integrated and plotted against known nisin concentration to obtain a standard curve. The nisin standard curve was used to calculate unknown nisin concentrations in the release solutions from corn zein films.

RESULTS & DISCUSSION
Antimicrobial activity of various films
The LBG and XG films collapsed due to their hydrophilic nature without forming a clear inhibition zone. CA films did not release enough nisin to produce a distinct inhibition zone. Further experiments demonstrated that the gelling kappa and iota CA did not show any release, while non-gelling lambda CA exhibited some nisin release. There is a possibility that nisin was physically entrapped in the gel matrix formed by kappa or iota CA, while it diffused readily through the non-gelling lambda CA. This absence of nisin release from kappa CA matrix has been attributed to the ionic binding of cationic nisin by anionic CA (several -OSO₃ groups) [3]. It has been reported that when a hydrophilic matrix comes in contact with water, it swells due to hydration, forms a gel layer that acts as a diffusion barrier, and impedes subsequent release of active compounds [10].

HPMC, a water soluble biopolymer, formed transparent and flexible films that completely dissolved on agar plates. To improve the physical integrity and nisin release, blended films were made with kappa CA and HPMC. When these HPMC/kappa CA blended films contained nisin, they exhibited significant nisin release, which increased as the content of HPMC in the matrix increased (Fig 1).

Figure 1: Antimicrobial activity of nisin containing films made with various blends of HPMC & kappa carrageenan.
For AL films, the inhibition zone decreased with increasing crosslinking time and % CaCl\textsubscript{2} (Fig 2), probably because the alginate gel strength increased. A stronger gel physically entraps nisin, thus preventing its release into the surrounding medium. Similarly, the size of the inhibition zone increased as the content of guluronic acid in the alginate decreased (Fig. 2). A higher porosity has been observed in alginate matrixes containing higher amounts of guluronic acid, which in turn, resulted in higher nisin release. Thus, to develop controlled release films with long-lasting and sustained release of nisin, it seems that alginates with lower guluronic acid content, longer crosslinking time and higher CaCl\textsubscript{2} content are the best choice.

<table>
<thead>
<tr>
<th>Type of alginate</th>
<th>Crosslinking time = 15 min</th>
<th>Crosslinking time = varying</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CaCl\textsubscript{2} = varying</td>
<td>CaCl\textsubscript{2} = 2.5%</td>
</tr>
<tr>
<td>High guluronic acid (67%)</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Low guluronic acid (34%)</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Figure 2:** Antimicrobial activity of nisin containing alginate films with various crosslinking times, % CaCl\textsubscript{2} and % guluronic acid

CZ films exhibited clear nisin activity by producing distinct inhibition zones, whose size increased with higher Nisaplin\textsuperscript{®} content (Fig 3).

**Figure 3:** Antimicrobial activity of corn zein films containing various amounts of Nisaplin\textsuperscript{®} (mg).
Effect of corn zein concentration on nisin release kinetics

The release profiles of CZ films indicate that nisin release decreased as the concentration of corn zein in the film matrix increased (Fig. 4). This was probably due to the increasing tortuosity of the film as the corn zein concentration increased. A Weibull model was developed with an excellent predictive power ($R^2 > 0.95$, see Fig. 4).

As corn zein concentration increased from 4 to 10% (w/v), a significant increase in the Weibull scale parameter, $\alpha$, was observed (from 6.7 to 25.2), indicating that the initial release was faster at lower corn zein concentrations. The value of the Weibull shape factor, $\beta$, was 0.49, 0.59 and 0.48 for CZ concentration of 4, 6 and 8% (w/v), respectively, indicating Fickian diffusion [11]. Films with 10% corn zein had a $\beta$ value of 0.9, which indicates a combined mechanism of Fickian and non-Fickian, case II transport. At 10% (w/v) corn zein concentration, the release could be governed by nisin concentration gradient or some other mechanism. After about 40 h of release, nisin concentration was similar for films with 8 and 10% corn zein, indicating that the effect of corn zein concentration on nisin release is more evident during the early part of diffusion and at low zein concentrations.

CONCLUSION

Hydrophilic biopolymers such as XG and LBG films were not suitable candidates for controlled release applications in aqueous food systems. Overall, HPMC, AL and CZ films showed good potential as a controlled release matrix. Alginate with lower guluronic acid content can be used as a controlled release matrix. Nisin release from CZ films can be controlled by varying the concentration of zein in the matrix, and the release kinetics can be predicted by Weibull based models.

Figure 4: Experimental (symbols) and Weibull model predicted (lines) nisin release from films with various corn zein concentrations
REFERENCES