Development of Model Active Packaging System and Inactivation of Surface-Associated Listeria monocytogenes by Controlled Release of Nisin

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ABSTRACT: We introduce a simple model food/packaging system to study the efficacy of controlled antimicrobial agent (AMA) release on the bacterial growth inhibition. The system permits considering the effect of headspace, either liquid- or gas-filled. Both types of headspace showed significant decrease in the bacterial growth inhibition due to limited AMA transport from the packaging layer to the model food. The model food/packaging system has been validated on a ready-to-eat meat product (sliced turkey), and reasonable bacteria inhibition levels were achieved.

THE food market has growing demand for fresh and minimally processed foods. However, these foods are highly perishable and more susceptible to microbial spoilage. Thus, there is a strong need to develop new preservation methods to achieve a required level of safety, quality and nutritional value of food during extended shelf life period. The use of active packaging (AP) materials is one of the post-processing methods to preserve food products and meet consumers' expectations.

Antimicrobial packaging is designed to control microbial growth in a food product. It consists of an antimicrobial agent (AMA) immobilized onto internal surface of a package or incorporated into packaging material (Han and Floros, 1998). In the latter case AMA is released into a food product over time. This permits to extend shelf life of food products, helping to reduce the amount of AMA in food formulation.

AP materials have many parameters that influence their antimicrobial efficacy, which is thoroughly addressed in a number of studies: polymer processing, polymer morphology (Petersen et al., 1999), polymer swelling (Buonocore et al.) and AMA affinity to the packaging material (Soliva-Fortuny and Martin-Belloso, 2003). The food product was considered in all these papers as a homogeneous medium in full contact with the packaging. However, surface morphology of the foods is an important parameter that determines mass transfer of an antimicrobial agent through the interface between packaging and food.

VARIOUS TYPES OF FOOD/PACKAGING CONTACTS

Based on the nature of a food product and corresponding morphology of a food surface one can distinguish five types of food-packaging contacts depicted in Figure 1.

If the surface of a food product is flat, direct contact between the packaging and the food exists. This AP system has maximum efficacy. An irregular food surface will cause only partial contact between the packaging material and the food product, developing non-continuous headspace. The headspace configuration influences AMA transport from the packaging to the food. Depending on the dominating physical state of a food product, the packaging surface can be in contact with solid or with liquid food products, or sometimes both. These contacts can be direct or indirect if headspace exists between the food surface and packaging material (see Figure 2). Depending on the type of the food product, the headspace can be liquid- or gas-filled.

Numerous parameters can influence the efficacy of AP, including the packaging material properties, the antimicrobial transport, the bacterial population response and the food matrix. Most of the published studies investigated antimicrobial activity of the controlled release compound by adding AMA directly to the foods

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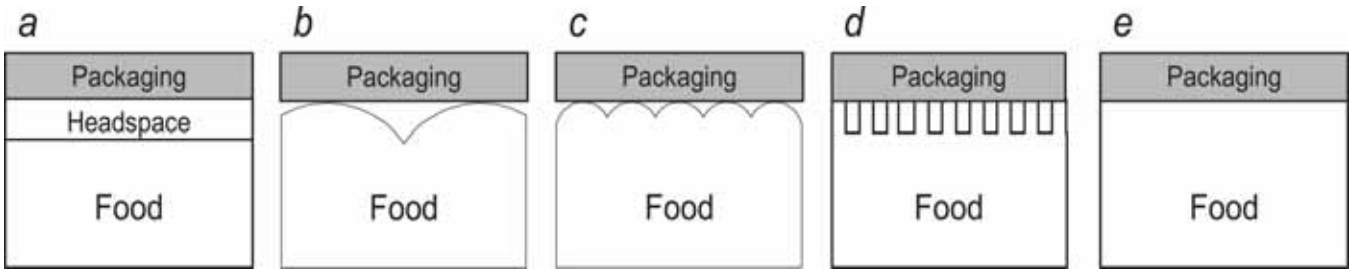

Figure 1. *Spatial organization of AP as a function of food surface morphology: direct contact (a), partial contact ~1 cm (b), ~1 mm (c), ~10 μm (d) and direct contact (e).*

or to the packaging materials. However, these two methods have significant disadvantages:

- When an AMA is added directly to the food, experimental data provide will important information on the antimicrobial activity of AMA and its interaction with the food matrix. There is no time-dependent AMA release; therefore these studies are insufficient for the development of AP.
- On the other hand, if AMA is incorporated into the packaging material, there is no control over the antimicrobial release. The effects of packaging material properties on the AMA release rate cannot be distinguished from the effects of the AMA release rate on the bacterial inhibition.

There is a need to understand bacterial response to the AMA release without the influence of material-dependent properties of AP. No standard method has been established to investigate the effect of antimicrobial agent's time-dependent release on the bacterial response. This study aims to design a method that will link microbiological studies to packaging design by developing a model food/packaging system with controllable release properties and flexible configuration.

MATERIALS

Model Microorganism

Listeria monocytogenes strain 10403, a human clinical isolate, serotype 1/2a (purchased from Dr. Portnoy, University of California, Berkeley) was used to test the efficacy of AP model. The stock culture was maintained at –80°C in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, MI) with 20% Glycerol. The cells were stored on BHI agar (Difco) at 4°C and sub-cultured every two weeks in BHI broth at 30°C for 20 hours. Before each experiment the cells were brought to mid-log phase by adding 1 ml of the subculture to 9 ml of BHI broth and incubating at 30°C for 4 hours.

Antimicrobial Agent

Nisin—the bacteriocin produced by *Lactococcus lactis*—has been shown to be effective against *L. monocytogenes*. Nisin creates pores in the membranes of Gramm-positive bacteria and causes the dissipation of transmembrane potential, creating collapse of the proton motive force and the lysis of the cell (Szabo and

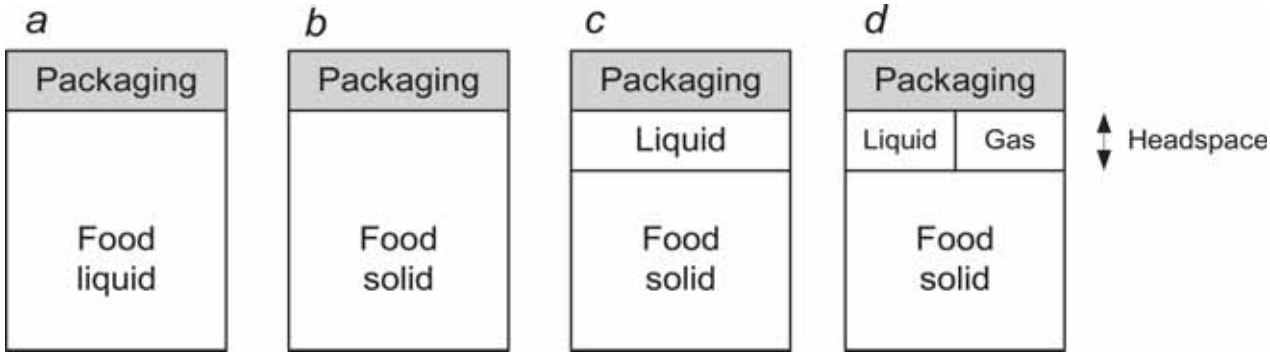

Figure 2. *Dependence of a food/packaging interface on a type of food product.*

Cahill, ; Thomas et al., 2002). It is the only bacteriocin generally recognized as safe in the US for use in processed cheese spread and it is approved for use in selected foods in more than forty countries (Abee and Wouters, 1999). Commercial-grade nisin (nisalpin) purchased from Sigma Chemical Co. (St. Louis, MO) was dissolved in sterile water and adjusted to pH 2 with hydrochloric acid. A fresh solution of 10,000 IU/ml was prepared before each experiment.

Determination of Nisin Activity

L. monocytogenes was inoculated to get an initial cell concentration 10^5 cfu/ml. Immediately after inoculation the samples were incubated at 30°C and the growth was assessed over 48 hours by absorbance measurements using the MRX II microplate reader (Dynex Technologies, Chantilly, VA).

After 24 hours exposure to different nisin concentrations 20 µl of each culture were re-suspended in 10 ml fresh BHI broth with no nisin. The samples were incubated at 30°C and the growth was assessed over 24 hours by optical density reading using the microplate reader.

Model Semi-Solid Food Product

Since most of listeriosis outbreaks are associated with the surface contamination of ready-to-eat meat products, our food model should represent this type of surface. The food model selected for this system was Tryptic Soy Agar (TSA, Difco), because it has been shown to be a good surrogate of meat surface (Midelet and Carpentier, 2002). Prepared TSA has a final pH of 7.3, and an agar concentration of 15 g/L.

Tested Food Product

Sliced lean white turkey, oven-roasted style (Butterball, ConAgra Foods, Omaha, NE) was used in this research; its pH = 6. To quantify bacterial load, a total plate count (TPC) assay was performed by stomaching 25 g of sliced turkey in 225 ml of 0.1% peptone water for 1.5 minutes, and plating dilution 10° to 10^{-4} on TPC agar (Difco). No growth has been observed after incubation of the TPC plates at 30°C for 48 hours.

Model Material for Active Packaging

Agar is a porous solid matrix that permits easily control the antimicrobial release rate. The diffusion coefficient of nisin into 3% agarose has been determined by (Sebti et al., 2004). It has been estimated to be a 8.14×10^{-11} m^2/s. They have also shown that the nisin concentration in gel does not influence the diffusion process. Nisin diffusivity in agar remains constant until agarose concentration reaches 8%.

Agar (Difco) was used as an entrapment matrix for nisin. Agar was dissolved to obtain a 2% solution and autoclaved at 121°C for 15 minutes. Prepared agar was poured into Petri dish to form a layer. Changing the amount of agar one can obtain model packaging materials with various thicknesses.

Evaluating the Effect of AMA Load on Bacterial Growth

To evaluate the influence of antimicrobial agent load five model packaging layers with predetermined concentrations of nisin (0, 10, 100, 500, and 1000 IU/ml) were prepared. *L. monocytogenes* culture was inoculated on the surface of TSA plates to obtain overall concentration of 100–300 cfu/plate. The agar "packaging" layers were placed on top of inoculated TSA surfaces [see Figure 3(a)]. The resulting system consists of surface-contaminated meat surrogate (TSA) and controlled-release (active packaging) material in direct contact with contaminated food.

Evaluating the Effect of Air Filled Headspace on the Efficacy of AMA Release

To investigate the effect of air filled headspace, samples were prepared with inoculated TSA and nisin layers of 0, 10, 100 and 1000 IU/ml. Air gaps were created at the periphery of the plates, so there was direct contact between nisin-containing layer and the "food" layer at the center of the plate, and the air-filled headspace surrounding the direct contact area [see Figure 3(b)]. The plates were incubated at 30°C for 36 hours; bacterial growth was assessed by direct colony count in the areas of direct contact and in the regions with the air-filled headspace.

Evaluating the Effect of Liquid Filled Headspace on the Efficacy of AMA Release

For the experiments with liquid-filled headspace both TSA and turkey slices were used as target food surfaces. The AMA loads in the nisin layer were 0, 100 and

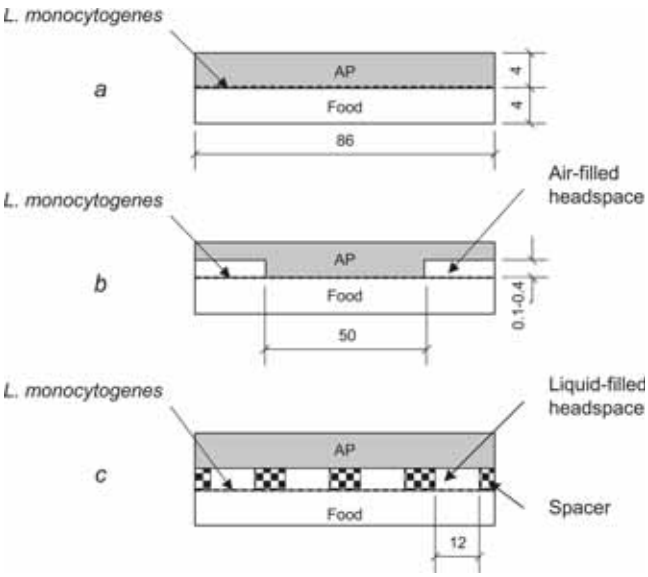

Figure 3. *Food packaging model system (all dimensions in mm).*

1000 IU/ml. To create the headspace we have made a spacer from nylon mesh and placed it between the model food and "packaging" layer [see Figure 3(c)]. To mimic headspace liquid, 8 ml of 0.1% peptone water was poured over the food surface. Samples were incubated at 30°C for 36 hours; stomached and plated on Modified Oxford medium. Direct colony count was performed after appropriate incubation period as described above.

RESULTS

Design of the Model Food/Active Packaging System

The first objective of the study is to design a system that will deliver an AMA with continuous release. The packaging material should not be subject to swelling. It should be favorable to bacterial growth (by not adding supplementary stress), but it should not enhance the growth by, for example, providing extra nutrients. The properties of the designed model system will be similar to AP, but the effect of the packaging material matrix on the release process will be controllable.

A model food/packaging system was designed in the way to obtain homogeneous distribution of AMA within the packaging matrix, and insure controllable release of the stored antimicrobial to the food. We utilize a sandwich-type design of the model system, see Figure 3. The bottom layer is the food product (model or real) with surface inoculated by *L. monocytogenes*. The top layer consists of agar with entrapped antimicrobial agent molecules. Agar layer used as the model packaging matrix has three parameters that can be used to control nisin release rate: viscosity, thickness, and AMA load.

Diffusion of Nisin from Plane Surface AP into Semi-Infinite Food Medium

Let us consider a food/packaging system as depicted in Figure 3. AMA diffuses through the polymer matrix towards the interface between packaging and food. This process is driven by concentration difference between the packaging and the food product. In case of amorphous polymer matrix above its glass transition temperature diffusion mechanism of an AMA can be described by Fick's law, i.e. AMA concentration change with time is proportional to the rate of antimicrobial agent concentration gradient change with distance:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad (1)$$

A common assumption in the packaging literature is that the diffusion is unidirectional and diffusion coefficient is constant within each material layer (packaging, headspace, and food). The diffusion rate is determined by the properties of the polymer matrix, interaction between AMA and packaging material (solubility of the AMA in the polymer and interaction forces between the AMA and polymer molecules), and environmental factors: temperature, pressure and the composition of the food matrix. The antimicrobial agent migrates from the package across the interface between the polymer matrix and the food. When packaging material is not in the direct contact with the food product, AMA migration through this headspace can be a rate-limiting step for the whole delivery process.

It is clear that the diffusivity of an antimicrobial agent wouldn't be a rate-limiting step for AMA release, since the viscosities of the food product and packaging material are much higher than that for the headspace-filling substance. Even more, if headspace is thin enough it will not affect the overall delivery rate of the released agent. In this case the migration time of antimicrobial compound through the headspace is much smaller than the characteristic migration (diffusion) times for the packaging material and food product, and obviously, the shelf-life period. The last requirement is important

for large food products ($L_f \approx l_p$, where L_f, l_p are the characteristic size of a food product and the thickness of an AP material correspondingly), that can be considered as semi-infinite media. It is well known that the characteristic diffusion time scale is $\sim l^2/D$. Therefore, "no headspace impact" condition can be written as:

$$\delta \approx \min\left[l_p \sqrt{\frac{D_\delta}{D_p}}; \sqrt{D_\delta t_{sl}}; L_f \sqrt{\frac{D_\delta}{D_f}} \right] \qquad (2)$$

where D_δ, D_p, D_f are the diffusivities of AMA in the headspace medium, packaging and food respectively; δ—the headspace thickness; t_{sl}—the shelf-life period or total time of experiment.

The typical diffusion coefficient for packaging materials is about 10^{-12} . . . 10^{-15} m^2/s, and the thickness is ~100 … 400 μm. Limiting condition (2) is valid for liquid-filled headspaces with thicknesses up to ~500 μm. However, for gas-filled headspaces requirements (2) are difficult to satisfy due to the low mobility of AMA in the gas phase.

If a liquid-filled headspace in a food/packaging system satisfies (2) it can be excluded from the model, since it does not impact diffusion-controlled transport of released antimicrobial agent. This food/packaging system is analogous to that depicted in Figure 1(e). It can be represented as a semi-infinite medium, i.e. a homogeneous food product with a thin surface layer where the transport properties differ from those of the rest of the medium. The boundary conditions at the food/packaging interface ($x = 0$) are continuity and "no accumulation":

$$\begin{aligned} C_p &= C_f \\ D_p \frac{\partial C_p}{\partial x} &= D_f \frac{\partial C_f}{\partial x} \end{aligned} \qquad (3)$$

Following the method described in (Crank, 1975), the solution of Equation (1) with boundary conditions at the food/packaging interface, zero initial concentration of AMA in the food, and initial concentration of antimicrobial agent C_{p0} loaded into the packaging material can be written in the form:

$$C^*(t) = \frac{1}{2} \operatorname{erfc} \frac{1}{2\sqrt{\mathrm{Fo}}} \qquad (4)$$

where,

$$C^*(t) = \frac{C_f(t)\big|_{x=0}}{C_{p0}}$$

and

$$F_o = \frac{tD_p}{l_p}$$

is the Fourier number of a packaging material.

One should note that there is a difference between the model gel-type AP material used in our experiments and a real food/packaging system. The model packaging material and food product studied both have similar transport properties ($D_p \sim D_f$), while for the majority of "real life" packaging materials these properties are quite different $D_p \sim D_f$. We can use the solution (4) to analyze the model food/packaging system in terms of controlled release of the active compound. (Sebti et al., 2004) studied nisin diffusion in 3% agarose gel. The diffusion coefficients at 5.4 and 22.3°C were found to be equal to 1.92 and 8.14 × 10^{-11} m^2.s^{-1} respectively. The diffusion coefficients in agar can be assumed to be of the same order.

It is clear that for surface-contaminated food products homogeneous active packaging material provides a bi-modal AMA delivery. For the initial period of time (~12 hr) the AMA surface concentration is almost constant and, therefore, its action is similar to the formulation-based AMA delivery. For extended shelf-life period the concentration of AMA exponentially decreases. Therefore, the microbial population responses to the contact with active packaging material significantly differ for long- and short-time shelf life periods.

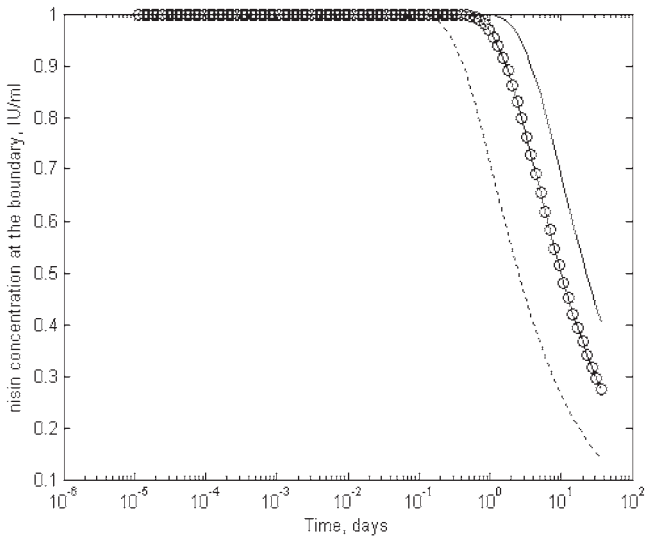

Figure 4. *Nisin concentrations at the agar surface with initial nisin concentration of 100 IU/ml and for the agar layer thicknesses: — 2 mm, ○ 4 mm, and - - 6 mm.*

Direct Contact Mode: Concentration of AMA Incorporated into the Agar Matrix Affects Bacterial Growth

Once the system was designed, the first step was to vary one of the parameters: the concentration of nisin incorporated into the packaging matrix to obtain inactivation kinetics with different nisin release profiles and compare the results with the standard method. When the nisin concentration in agar layer is high, the AMA release rate should increase influencing bacterial inhibition. We have used relatively small concentrations of nisin because of the consumer demand for food products with minimal amount of additives. Additionally, all active packaging materials can be characterized by very high retention rates with the total amount of released antimicrobial not exceeding 5% of its actual load. All samples in this experiment were made by inoculation of TSA surface by *L. monocytogenes* 10^2 cfu/plate with following incubation for 36 hours at 30°C. The only variable in this experiment was the nisin concentration that was varied from 0 to 1000 IU/ml.

To separate the effect of the model packaging material (agar) and AMA we have performed a control experiment comparing TPC values for inoculated TSA plates uncovered and covered with agar layer with no nisin added. The "food" sample covered with agar layer had bacterial counts 190 cfu/dm^2, while uncovered "food" sample had 230 cfu/dm^2, which is a normal error for plate counting method. Hence, agar "packaging" layer does not affect bacterial growth. All observed changes in bacterial growth are, therefore, due to presence of nisin in the packaging layer.

One should note that bacterial colonies developed under the agar layer were much larger than the colonies on the surface without agar. This can be explained by lower oxygen availability under the agar layer. (Nilsson et al., 1997) showed that *L. monocytogenes* growing under 100% CO_2 atmosphere had 2–5 times more elongated cells. This suggests that the changes in the cell morphology can be responsible for the shape of colonies that are formed under limited oxygen supply.

Figure 5 shows data on bacterial survival obtained in the agar packaging layers with various nisin concentrations. The layer containing 10 IU/ml of nisin provides 40% inhibition compared to the 0 IU/ml control sample. The 100, 500 and 1000 IU/ml samples showed significant (100-fold) bacterial inhibition. The maximum inhibition effect was observed for 500 IU/ml.

The results observed are consistent with the operational mode of the system designed. As the AMA load increases in the "packaging" layer, the nisin release rate also increases due to higher concentration gradient across the food-packaging interface. The increased release rate enhances microbial inactivation. The relatively low effect of packaging layer with low nisin content can be explained by *L. monocytogenes* tolerance of nisin and the existence of sublethal bacteriocin dose.

The 10 IU/ml nisin load had little effect on the bacterial growth, but concentrations above 100 IU/ml had considerable effect. The bacterial growth observed at

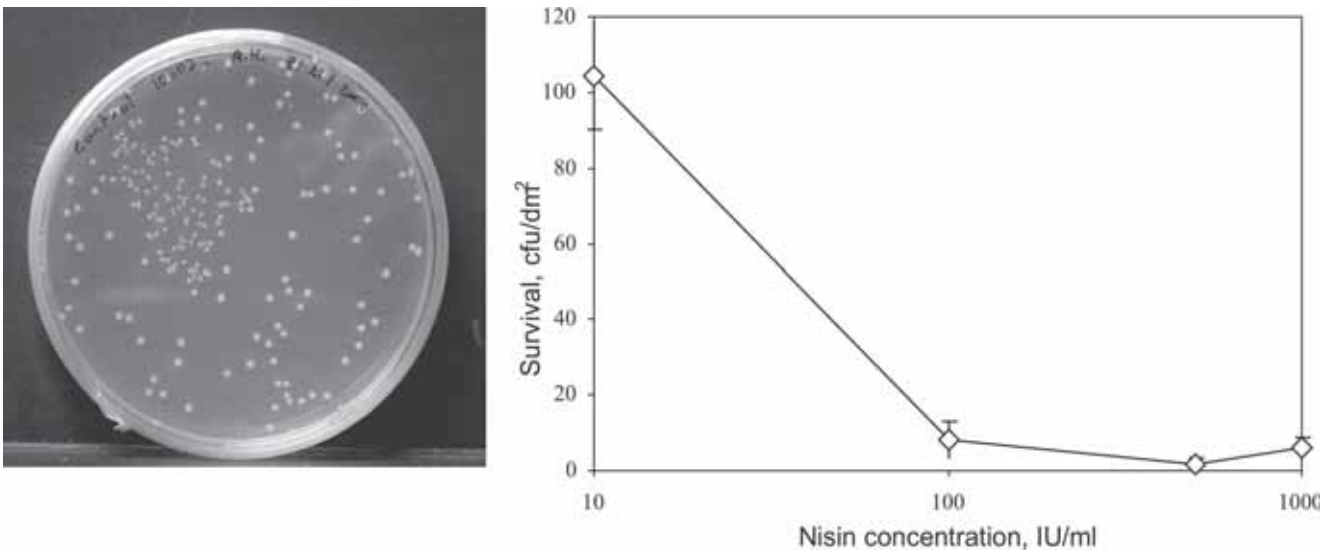

Figure 5. *Inhibition of* L. monocytogenes *under AP layer in direct contact with the model food: a)* Listeria *colonies under agar layer containing 100 IU/ml; b) bacterial survival as a function of nisin load of the AP layer.*

the nisin load of 1000 IU/ml could be explained by either stress adaptation or nisin-resistant bacterial mutants development, as described by (Chi-Zhang et al., 2004).

Effect of Air Filled Headspace on the Efficacy of Antimicrobial Controlled Release

The efficacy of antimicrobial packaging is affected by the presence of an air-filled headspace between the food surface and the package. It is often observed for irregularly-shaped foodstuff (e.g. vegetables, meet, etc.). The food/packaging model developed allows for investigation of the headspace effect.

We have designed the experimental setup so that a "ring of air" (i.e. gas-filled headspace) has been formed within the Petri dish along its perimeter [see Figure 6(a)]. Therefore, our model system contained two distinct regions with different AMA release conditions: a "direct contact" food/packaging zone with the agar layer in the center of the plate, and a "non-contact" zone at the periphery. The width of the air-filled "ring" (a) has been chosen to be $a = R(1-1/\sqrt{2})$, so the areas of direct contact zone and air-filled headspace were equal. Samples were prepared with 0, 10, 100, 500, and 1000 IU/ml loads of nisin.

The control sample with the agar layer containing no nisin had 190 cfu/dm^2 in the area of direct contact zone, and 118 cfu/dm^2 under the air-filled headspace. The results depicted in Figure 6(b) show that there was no significant inhibition of bacterial growth under the air filled headspace. Thus, the antimicrobial efficacy of the packaging is significantly reduced by the presence of gas-filled headspace due to low gas mobility of antimicrobials. Accordingly to Graham's law, molecular mobility of substances in gases is:

$$D \propto \frac{1}{\sqrt{MW}} \tag{5}$$

where MW is the molecular weight of the substance. Since the molecular weight of nisin is 3354.07, its mobility in the air is ~10.6. times lower than that of oxygen.

Liquid-Filled Headspace and the Efficacy of the Active Packaging

Many packaged food products contain a liquid-filled headspace. This headspace can exist in two cases: juice/liquid naturally extracted from the product as a result of its processing or storage; and liquid added to the product for food preservation and/or conditioning. The presence of liquid in the headspace could limit the transport of AMA from the package to the food.

The sliced turkey food sample was used for this experiment. The headspace was created by a spacer made of nylon mesh, filled with peptone water. The agar layer had nisin concentrations of 0, 100, or 1000 IU/ml. Bacterial growth levels were measured after 36 hours of incubation at 30ºC.

The results are displayed in Figure 7. As it was expected, the samples with headspace had higher levels of

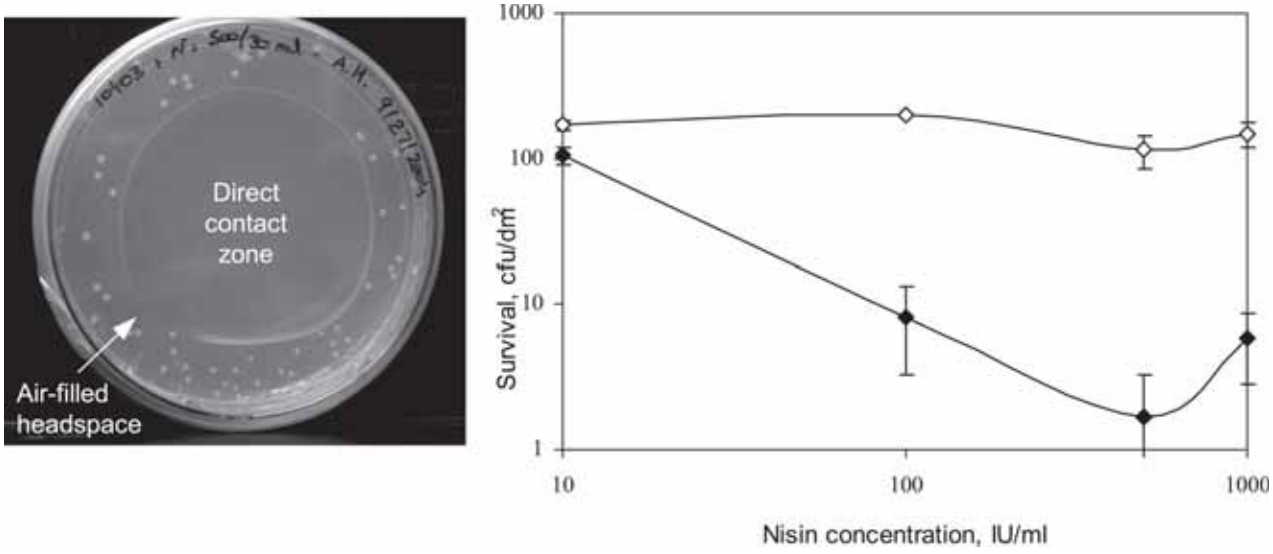

Figure 6. Colonies formed on TSA for the sample with 500 IU/ml nisin in the agar layer (left), and survival of* L. monocytogenes *(right): ◇—under the air filled headspace, ●—under the agar layer with various concentrations of nisin.

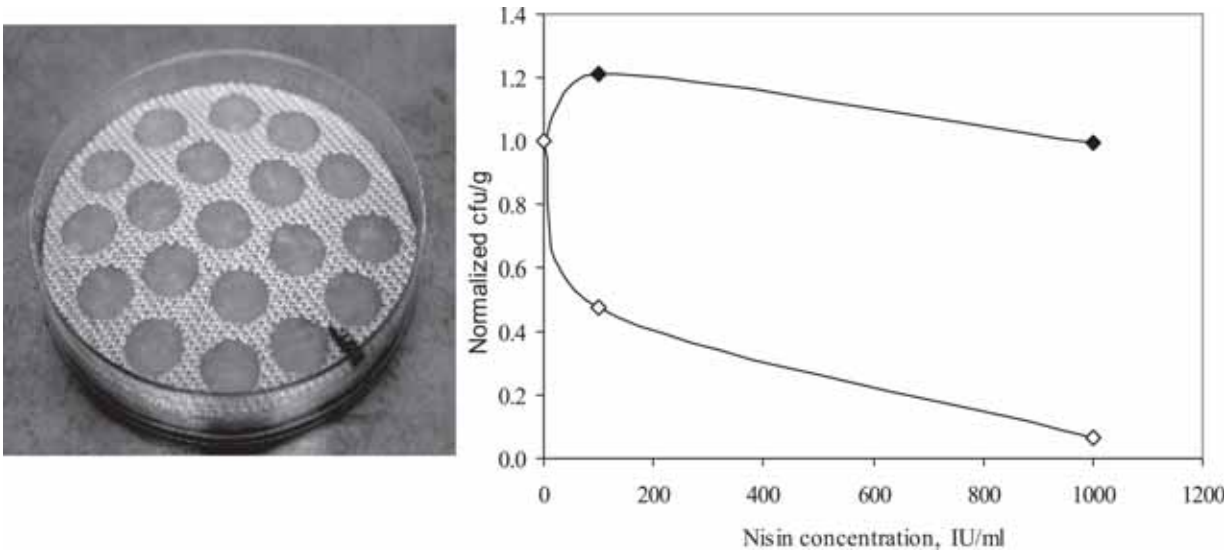

Figure 7. Nylon spacer used to model liquid headspace (left), and growth of* L. monocytogenes *on sliced turkey (right) at various concentrations of nisin: ◆—with the liquid-filled headspace, ◇—under the agar layer with no headspace.

bacterial growth than the samples with no headspace. The growth in the 1000 IU/ml sample with headspace was higher by almost one fold compared to the sample with no headspace.

These results show that headspace decreases the level of bacterial inhibition, probably because it limits the AMA transport from the packaging to the food surface. Therefore, the presence of liquid-filled headspace and its effect on the inhibition of bacterial growth should be taken into account when studying the efficacy of active packaging.

DISCUSSION

A model food/packaging system has been developed to investigate material independent AMA release efficacy. The agar matrix allows controllable and homogeneous release of the AMA; the release rate of the active compound can easily be quantified using the mathematical model developed. The system has been tested on TSA and model food product; the bacterial growth inhibition has been quantified by direct plate counting. Consistent inhibition levels have been observed with nisin concentrations tested, and good correlation was obtained with the standard agar diffusion test.

Depending on the environment and on the nisin load, some bacteria can develop resistance to the antimicrobial agent. The change in their sensitivity is due to changes in the fatty acid composition of the membrane of the resistant cells (Mazzotta and Montville, 1997). Numerous factors can influence the development of nisin-resistant bacteria: the dose of nisin, the method of its application, combination with other treatments, etc. Development of the mutants explains observed overgrowth of *L. monocytogenes* at high AMA loads (see Figure 5 and Figure 6).

One can recognize two characteristic timescales for antimicrobial delivery through the packaging headspace δ : diffusion migration time

$$t_{diff} \approx \frac{\delta^2}{D} \tag{6}$$

where D is AMA diffusivity, and characteristic time for bacteria reproduction:

$$t_b \approx \frac{1}{\mu} \tag{7}$$

where μ is the growth rate of bacteria.

Therefore, to prevent bacterial growth, AMA should be delivered through the packaging headspace faster than bacterial population growth. In other words:

$$t_{diff} < t_b \tag{8}$$

One can estimate a critical thickness for the headspace as following:

$$\delta_{cr} = \sqrt{\frac{D}{\mu}}$$

If $\delta < \delta_{cr}$ headspace has no effect on the effectiveness of antimicrobial control release from the packaging material and packaged food can be considered in direct

contact with packaging. Increase of headspace ($\delta > \delta_{cr}$) results in delayed delivery of AMA and decreased efficacy of inhibition. This means that to inhibit bacterial growth one will need to deliver increased amount of antimicrobial agent, which results in higher processing costs and lower food quality.

CONCLUSION

We have introduced a simple model food/packaging system to study the efficacy of controlled AMA release on bacterial growth inhibition. The system designed permits considering the effect of headspace, either liquid- or gas-filled. Both types of headspace showed significant decrease in the bacterial growth inhibition due to limited AMA transport from the packaging layer to the model food. The model food/packaging system has been validated on a ready-to-eat meat product (sliced turkey), and reasonable bacteria inhibition levels were achieved. The influence of headspace, food matrix and packaging design can be tested with this system, which provides important information for the development of active packaging and characterization of antimicrobial release.

REFERENCES

Abee, T., and J. Wouters. 1999. Microbial stress response in minimal processing. *International Journal of Food Microbiology* 50(1–2):65–91.

Buonocore, G.G., M. Sinigaglia, M.R. Corbo, A. Bevilacqua, E. La Notte, and M.A. Del Nobile. Controlled release of antimicrobial compounds from highly swellable polymers.

Chi-Zhang, Y., K.L. Yam, andM.L. Chikindas. 2004. Effective control of Listeria monocytogenes by combination of nisin formulated and slowly released into a broth system. *International Journal of Food Microbiology* 90(1):15–22.

Crank, J. 1975. The mathematics of diffusion. Clarendon Press, Oxford.

Han, J.H., and J.D. Floros. 1998. Simulating diffusion model and determining diffusivity of potassium sorbate through plastics to develop antimicrobial packaging films. *Journal of Food Processing & Preservation* 22(2):107–122.

Mazzotta, A.S., andT.J. Montville. 1997. Nisin induces changes in membrane fatty acid composition of Listeria monocytogenes nisin-resistant strains at 10°C and 30°C. *Journal of Applied Microbiology* 82(1):32–38.

Midelet, G., and B. Carpentier. 2002. Transfer of Microorganisms, Including Listeria monocytogenes, from Various Materials to Beef. *Appl. Environ. Microbiol.* 68(8):4015–4024.

Nilsson, L., H.H. Huss, and L. Gram. 1997. Inhibition of Listeria monocytogenes on cold-smoked salmon by nisin and carbon dioxide atmosphere. *International Journal of Food Microbiology* 38(2/3):217–227.

Petersen, K., P. Vaeggemose Nielsen, G. Bertelsen, M. Lawther, M.B. Olsen, N.H. Nilsson, and G. Mortensen. 1999. Potential of biobased materials for food packaging. *Trends in Food Science & Technology* 10(2):52–68.

Sebti, I., D. Blanc, A. Carnet-Ripoche, R. Saurel, and V. Coma. 2004. Experimental study and modeling of nisin diffusion in agarose gels. *Journal of Food Engineering* 63(2):185–190.

Soliva-Fortuny, R.C., and O. Martin-Belloso. 2003. New advances in extending the shelf-life of fresh-cut fruits: a review. *Trends in Food Science & Technology* 14(9):341–353.

Szabo, E.A., and M.E. Cahill. Nisin and ALTA 2341 inhibit the growth of Listeria monocytogenes on smoked salmon packaged under vacuum or 100% CO2.373–377, 1999 May.

Thomas, L.V., R.E. Ingram, H.E. Bevis, E.A. Davies, C.F. Milne, and J. Delves-Broughton. 2002. Effective use of nisin to control Bacillus and Clostridium spoilage of a pasteurized mashed potato product. *Journal of Food Protection* 65(10):1580–1585.