

Coating Trial of an Antimicrobial Coating Containing Nisin Using a Large Scale Gravure Application Process

M. Perna, D. Darby, P. Gerard, J. Rhodehamel and K. Cooksey*

*Clemson University

Department of Food, Nutrition and Packaging Sciences, B221 Poole & Agricultural Building,
Clemson University, South Carolina, 29634, USA

Email: mperna@g.clemson.edu Phone Number: 864-640-9340

Abstract

Nisin is a GRAS (generally recognized as safe) approved antimicrobial peptide that has been found to be effective against Gram positive microorganisms. Implementation of nisin into antimicrobial packaging has the potential to extend product shelf life through inhibition of spoilage microorganisms and appeal to industry as a value added packaging product. This study found that it is possible to produce an antimicrobial coated material using gravure production processes but not without difficulties. The coated material produced resulted in adhesion difficulties; however a second trial will be conducted using flexography after troubleshooting quality issues. The produced material maintained antimicrobial efficacy against *M. luteus* when control films were compared to treatment films. ($P < 0.0001$)

1.1 Introduction

Food spoilage is one of the major causes of food waste. Approximately 40% of food in the United States goes to waste this can include wasted food from production, distribution, retail and household environments. Of household foods in the United States, approximately 2/3 of products are lost due to spoilage. (NRDC 2013) Food waste and the consumers demand for fresh, minimally processed food products have caused an increased need for active packaging. Active packaging is increasing in demand due potential for the reduction of food waste through shelf life extension by reduction of spoilage microorganisms The active packaging sector is expected to grow to 3.5 billion dollars by 2017 in the United States and 17.3 billion dollars worldwide. (Spinner 2014)

Nisin is a GRAS approved natural antimicrobial peptide. Several studies have shown nisin to be effective in inhibiting gram positive bacteria showing potential in the food packaging market for the reduction of spoilage microorganisms. The cost inherent from the loss of product due to the growth spoilage microorganisms is a concern for many packaging companies. Antimicrobials are also typically a costly packaging addition. Application of nisin in a commercially available packaging product for food could be used for food product shelf life extension and appeal to industry as a value added product.

Few studies have been conducted on antimicrobial coated materials produced using large scale equipment such as gravure coaters and additional printing methods such as flexography. The main objectives of this study was to produce antimicrobial coated material containing nisin using a direct gravure coating process and to characterize both some key characteristics of the liquid coating and coated material produced.

1.2 Materials and Methods

Coating Preparation

Approximately two gallons of both control and treatment coating solutions were prepared for the gravure trial. Control coating batches did not contain Nisin (2.5%) component but contained all other coating ingredients. The coating solution was prepared by heating and simultaneously stirring 0.55 pounds of PVOH resin in 750 mL of distilled water for approximately 1-2 hours until the resin dissolved into solution. The hot plate stirrer was set to 175°C and the water/resin solution was stirred by hand with a wood spoon until later in the preparation process. Once the resin had dissolved, the solution was removed from the hot plate to allow slight cooling prior to adding 80 mL of glycerin (40 parts per 100 grams of PVOH resin) and 4.625 mL of Polysorbate 80 (0.25% v/v) (FCC, Spectrum Chemical Manufacturing Group, New Brunswick, NJ, USA). In a separate (1L) beaker, 25 gram of nisin containing commercial product (2.5% - 12,500 IU/mL in solution) (Danisco, Inc. Madison, Wisconsin, USA) was dissolved in 50 mL of 0.02 M acetic acid solution. (Franklin et al 2004) (Glacial acetic acid, Fischer Scientific, Waltham, MA, USA) 750 mL of 95% ethanol was then added. The solution was then mixed using a tissue homogenizer to achieve particle suspension. The ethanol solution was then poured into the resin solution and stirred using a stir bar on the hot plate stirrer for an additional 10-15 minutes. Each batch was poured into either a 2 or 4 liter bottles for storage prior to the trial. Parafilm® and foil was wrapped around the closure to reduce any evaporation of the coating while being stored prior to trials.

Material Surface Treatments and Preparation

The material to be coated was a multilayer, 2.5 mil thick; PET (polyethylene terephthalate) coextruded lidding material commonly used for hot dog packaging donated by Sealed Air Corporation. The sealant web of the material consisted of linear low density polyethylene (LLDPE).

The web was slit down to 14.5 inches per the specifications of coating/laminating equipment to be used for the trial. Leader material was added to the beginning and ends of the web for machine set up and parameter adjustments. The roll was then corona treated on an inline Vetaphone Corona-Plus corona treater on a OMET VaryFlex 530 press at a line speed of 150 ft/min at 1000 watt*min per m² to reach a desired 37 dynes/cm surface tension. A water soluble primer, polyethylenimine (PEI) primer was then added to the web prior to the coating trials.

Liquid Coating Characteristics

Percent solids were measured before and after the trial run. This could indicate solvent evaporation during storage or the trial process. Liquid coating was weighed into previously dried and weighed aluminum pans. The pans were placed in a 65°C drying oven for 5-7 days. (Lindberg/Blue M Gravity Oven, Model GO1330A, Industrial Laboratory Heaters, Asheville, NC, USA) The pans were re-weighed on an analytical balance and percent solids were calculated. (n = 60)

pH of the coating solution was tested utilizing a Thermo Fisher-Orion Star A211 pH meter. (Thermo Fisher Scientific, Inc. Waltham, MA, USA).

Viscosity was estimated using a Zahn #3 cup. Measurements were collected in triplicate prior to and after trials were completed. (n = 12)

Coating Trial

Control and antimicrobial containing treatment coating trials were conducted within the same morning. Control coating trial was conducted first in order to avoid contamination should the treatment trial had been conducted first. Percent solids, pH and viscosity measurements were taken just prior to the start of each trial. Trials were run using the conditions listed in Table 1 using the leader material (PET) and basis weights were taken in line to make sure laydown was being achieved.

Table 1. Coater/laminator equipment parameters for primer, control and antimicrobial coatings to LLDPE Coex material.

Conditions of Coater/Laminator in DuPont Lamination Laboratory			
Sample	Primer	Control	Treatment
Primary unwind material	48 ga PET/2.5 mil LLDPE Coex/ 48 ga PET		
Coat side	In	Out	Out
Tension (1° UW) (psi)	4	1.5	2.0
Web width (inches)	14.5	14.5	14.5
Rewind coat side	Out	Out	Out
Tension at rewind (psi)	10	10	10
Coater cylinder	200 Quad	110 Quad	110 Quad
Coating	Primer (PEI)	Control coating	Antimicrobial coating
Coating station tension (psi)	13	13	13
Dryer 1 temperature (°F)	155	155	160
Dryer 2 temperature (°F)	150	150	155
Line speed (ft/min)	26	25	25

Coated Material Characteristics

The coating weight or basis weight of the coating on the substrate was determined using ASTM 2217: Standard Practice for Coating/Adhesive Weight Determination. Approximately 25 feet of material was left un-primed in order to peel off control and treatment coatings for basis weight determination. (n= 21 per treatment)

Haze (ΔE) testing was conducted using a Minolta CR-400 chromameter (Konica Minolta, Tokyo, Japan). The colorimeter was calibrated using a white calibration standard and an untreated neat piece of LLDPE coex film. Measurements were recorded in triplicate from each coated or uncoated piece of film using the white calibration standard as a consistent background. (n=40) ΔE was then calculated using the following formula:

$$\Delta E: \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2}$$

Film on lawn testing was conducted against spoilage indicator organism *Micrococcus Luteus* which was propagated from -80°C freezer stocks. The bacteria was pulled from freezer stocks and streaked onto TSAYE plates (tryptic soy agar with yeast extract) and stored at 37°C

and incubated for 48-72 hours. The bacteria were then then transferred to 30 mL of TSBYE (tryptic soy agar broth with yeast extract) and incubated a second time prior to testing.

Film squares (1/2" or 12.7 mm) were cut from the rolls of film produced during the trial using a 1/2 inch sample cutter. Film on lawn was conducted by dipping a sterile swab into the working culture and swabbing the entire agar surface in the Petri dish. Treatment and control films were then placed coating side down onto the inoculated media. Zones of inhibition were then measured using a digital caliper in vertical and horizontal directions and averaged. (n=40)

Block testing was conducted on rolls produced from the coating trials. (n = 40; 20 per treatment) The blocks were produced at Bishop Branch Machine Work in Pendleton South Carolina according to the specifications in ASTM D3354-15: Standard Test Method for Blocking Load of Plastic Film by the Parallel Plate Method. The blocks were (4 in² surface area) of aluminum fitted for the SATEC T10000 Materials Testing System (Instron, Norwood, MA, USA).

Film samples approximately 4.5 in² in area and 2 layers in thickness were cut from the rolls. These samples were left to condition for 40-48 hours as noted in the ASTM standard. The testing procedure utilized from ASTM D3354-15 followed the constant rate of separation procedure. The blocks were separated at a rate of 0.2 inches per minute (5.1 mm per minute). Max separation was set to 0.75 in (1.9 cm). The max force (gf) for separation of the film layers was recorded.

1.3 Statistics

Statistics were conducted using SAS® On Demand for Academics SAS® Studio. Means and T-test procedures were conducted on pH, basis weight, haze, block testing and film on lawn datasets. Lsmeans or GLM procedures were run on viscosity and solids content data to determine interaction significance. Univariate procedure analysis was also conducted on the film on lawn datasets.

1.4 Results

Table 2. Summary of results for liquid coating and coated material characteristics.

Gravure trial testing summary results for coatings and coated films			
	Control	Antimicrobial	P values ($\alpha = 0.05$)
Solids content (%) (n = 60)	18.72±0.69	20.67±2.55	0.0002
Viscosity (sec) (n = 12)	BEFORE – 21.53±0.86 AFTER – 22.06±0.41	BEFORE – 20.10±0.72 AFTER – 17.67±0.12	<0.0001 C&T 0.0011 (treat* time)
pH (n = 11)	6.47±0.03	5.96±0.02	<0.0001
Basis Weight (#/ream) (n = 42)	1.50±0.13 (2.44±0.21 gsm)	1.48±0.20 (2.41±0.33 gsm)	0.7041

Block testing (gf) (n = 42)	290.60±94.86	321.35±52.89	0.2210
Haze (ΔE) (n = 40)	0.16±0.09	0.15±0.06	0.8675
Film on lawn (mm) <i>M. Luteus</i> (n = 21)	0±0.0	5.78±2.20	<0.0001

1.5 Discussion

Liquid Coating Characteristics

The addition of nisin in the treatment coating produced an increase in the Zahn cup time measurements indicating an increase in viscosity. This resulted in a significant difference between control and treatment coatings. ($P < 0.0001$). The percent solids of the treatment liquid coating solution was higher (20.67%) when compared to the control (18.72%) as expected with the addition of nisin to the treatment. ($P = 0.0002$) No interaction of the percent solids with time was found. The average pH measurements of the control and treatment coatings were both slightly acidic. The treatment coating (pH 5.96) was slightly more acidic than the control (pH 6.47) control coating. Utilization of acidic coatings on production equipment can degrade metal or polymer parts and tubing. It is recommended that thorough cleanings be implemented as a part of the manufacturing processes and to potentially implement corrosion resistant doctor blades, tubes and cylinders to account for the acidity and corrosiveness of the coatings.

Coated Material Characteristics

There was no significant difference in the coating laydown between the control (1.50 #/ream) and treatment (1.48 #/ream) coatings. This was expected as the gravure cylinder wells hold a specified volume of coating and the nisin added was not enough to cause a significant change in coating laydown using this process. ($P = 0.7041$)

Some degree of blocking was expected due to tackiness and blocking of drawdowns during preliminary work. The results from the block tested on average exceeded the limit of 200 gf state in the ASTM D3354 standard. There was no significant difference between the average blocking force measurements between the control (290.60 gf) and treatment (321.35 gf). ($P = 0.2210$) This could have been related to the dry ability of laying down 1.5 #/ream coat weight and the drying capacity of the tunnel dryers for this particular gravure system. There was a large degree of variation between the samples. This high degree of variation could have been due to the homogeneity of the coating on the film. It is possible that points of lower blocking could have been due higher concentrations of glycerin or polysorbate 80 (oily components) in a particular area versus areas with higher amounts of polyvinyl alcohol.

The results showed that ΔE calculations for control ($\Delta E = 0.16 \pm 0.09$) and treatment ($\Delta E = 0.15 \pm 0.06$) were not significantly different. ($P = 0.8675$) Values for ΔE of 1.0 or greater are changes in color difference that are perceptible to the human eye. (Upton 2005). Therefore the amount of nisin added to the treatment coating was not a large enough amount to cause a significant discoloration in the coated film compared to the control or uncoated film sample.

Film on lawn results showed that control and treatment samples containing nisin inhibited *M. luteus*. Treatment films produced zones of inhibition of 5.78 ± 2.20 mm which was significantly different compared to the control. ($P < 0.0001$) There were no inhibition zones extending further than the perimeter of the control films. This could have been due to potential inhibitory effects from the other ingredients within the coating. Polyvinyl alcohol is a highly swellable polymer. It may be possible that the polymers swellability when put in contact with moisture was desiccating or drying out the bacteria. Other ingredients within the coating such as ethanol and acetic acid solution were expected to evaporate during the process leaving residual or trace amounts in the dry film. It is possible that either of these components could have become trapped within the PVOH matrix and caused inhibitory effects when the coating matrix dissolved on the agar surface.

Coated Material Quality

The coated material produced from the gravure trial resulted in a lack of adhesion of the coating to the substrate. This also caused a lack of sealability which could have been due to excessive corona treatment or weak boundary layers produced by oily coating ingredients such as glycerin or Polysorbate 80. Upon unrolling film samples, the coating would delaminate from the substrate. This may have been due several potential aspects including excessive corona treatment, “skinning” due to lack of drying and poor primer application.

Excessive corona treatment has been found to cause sealing difficulties in LLDPE by producing a higher molecular weight in the affected area resulting in crosslinking thereby decreasing chain mobility and increasing the polymer melt temperature. (Farley and Meka 1994; Zhang, Sun and Wadsworth 1998) Lack of drying can also result in adhesion difficulties through a defect called “skinning”. This defect occurs when the upper layer of a coating is dried faster leaving the lower layer of the coating wet. Lastly, the application of the primer could have been affected by either improper application or dissolved by the wet coating due to lack of drying. It was determined that the gravure cylinder used to coat the primer was laying down excessive amounts of primer and could have been re-dissolving the primer in itself. (personal communication with Rob Hammond, MICA Corporation) It was recommended to use a 400-600 LPI cylinder to apply the correct volume of primer. Because this cylinder was not available for the gravure coater, other processes and capabilities were investigated for a second trial using a flexography press (OMET VaryFlex 530) located in the Sonoco Institute of Packaging Design and Graphics on Clemson University campus.

1.6 Conclusion

Based upon the research conducted, there is potential for producing an antimicrobial coated material containing nisin using large scale production equipment. However, there was quality issues with the material produced due to lack of coating adhesion to the LLDPE substrate. A second trial will be conducted in order to make adjustments for some of the issues previously discovered from the gravure trial. Regardless of adhesion issues, the material was able to maintain antimicrobial efficacy through agitation and slight heat abuse during the coating and drying processes. This shows that there is potential for this material to be utilized for inhibition of spoilage microorganisms. However there is much work to be conducted finalizing the material and testing on complex food systems rather than microbial agar.

1.7 References

1. **American Society for Testing and Materials (ASTM).** 2016. D1003-13. Standard Test Method for Haze and Luminous Transmittance of Transparent Plastics. West Conshohocken, PA, USA.
2. **American Society for Testing and Materials (ASTM).** 2015. D3354-15: Standard Test Method for Blocking Load of Plastic Film by the Parallel Plate Method. West Conshohocken, PA, USA.
3. **American Society for Testing and Materials (ASTM).** 2013. F2217-13: Standard Practice for Coating/Adhesive Weight Determination. West Conshohocken, PA, USA.
4. **Farley, J.M. and Meka, P.** 1994. Heat Sealing of Semicrystalline Polymer Films - III. Effect of Corona Discharge Treatment of LLDPE. *Journal of Applied Polymer Science*. 51:121-131.
5. **Natural Resources Defense Council [NRDC].** 2013. Saving Leftovers Saves Money and Resources. Retrieved 2 Feb 2016 from <http://www.nrdc.org/living/eatingwell/saving-leftovers-saves-money-resources.asp>
6. **R. Hammond.** 2015. MICA Corporation, personal communication.
7. **Spinner, J.** 2014. "Active/Intelligent packaging capturing global attention". Food Production Daily.com Retrieved from <http://www.foodproductiondaily.com/content/view/print880097>
8. **Upton, S.** 2005. Delta-E: The Color Difference. CHROMix ColorNews online reference. Retrieved 26 Jan 2016. From <http://www.chromix.com/colorsarts/smartNote.cxxa?snid=1145&-session=SessID:C615D346031481B520IOy42999AE>
9. **Zhang, D., Sun, Q., and Wadsworth, L.C.** 1998. Mechanism of Corona Treatment on Polyolefin Films. *Polymer Engineering and Science*. 38: 965-970.