



## Use of Lard, Food Grade Propylene Glycol, and Polysaccharides in Infused Nets to Control *Tyrophagus putrescentiae* (Schrank; Sarcoptiformes: Acaridae) Infestation on Dry Cured Hams

Xue Zhang<sup>1</sup>, Michael D. Byron<sup>1</sup>, Jerome Goddard<sup>2</sup>, Thomas W. Phillips<sup>3</sup>, and M. Wes Schilling<sup>1\*</sup>

<sup>1</sup>Department of Food Science, Nutrition and Health Promotion, Mississippi State University, Mississippi State, MS 39762, USA

<sup>2</sup>Department of Biochemistry, Molecular Biology, Entomology, and Plant Pathology, Mississippi State University, Mississippi State, MS 39762, USA

<sup>3</sup>Department of Entomology, Kansas State University, Manhattan, KS 66506, USA

\*Corresponding author. Email: schilling@foodscience.msstate.edu (M. W. Schilling)

**Abstract:** *Tyrophagus putrescentiae* (Schrank) is controlled in the U.S. dry cured ham industry with methyl bromide (MB) fumigation. However, MB fumigation is being phased out of use since it is an ozone depleting substance. The objective of this research was to evaluate ham nets that were infused with lard, propylene glycol (PG) and polysaccharide coating for their efficacy at controlling mite infestations on dry cured ham cubes from hams that were aged for 4 to 6 mo. Results indicated that fewer *T. putrescentiae* ( $P < 0.05$ ) were on ham cubes with treated nets containing PG when compared to the number of mites on ham cubes with untreated nets over 10 wk of storage. Lard infused nets without PG did not decrease the mite population ( $P > 0.05$ ). The net without coating slowed the growth and reproduction of *T. putrescentiae* since net controls had fewer mites ( $P < 0.05$ ) than controls without nets. Fungi were not present on ham cubes that were treated with PG-containing nets over 10 wk of storage with a few exceptions. In conclusion, lard and xanthan gum, or carrageenan + propylene glycol alginate treated nets containing the medium concentration of PG effectively inhibited mite reproduction and fungal growth on dry cured ham and could potentially be used in an integrated pest management program to control mites on dry cured hams.

**Keywords:** dry cured ham, gum, lard, mite reproduction, propylene glycol, *Tyrophagus putrescentiae*

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## Introduction

*Tyrophagus putrescentiae* (Schrank, 1781; Klimov and OConnor, 2010), known as the mold mite, cheese mite, or ham mite, is an ubiquitous and economically important mite species that infests a wide variety of stored foods, particularly those with high protein and

fat contents (Gulati and Mathur, 1995; Hughes, 1976; Sánchez-Ramos and Castañera, 2001). This mite is a major pest in nuts, grains, aged cheese, dried eggs, dry cured ham, dog food, mushroom beds, and mycology laboratory cultures (Arnau and Guerrero, 1994; Canfield and Wrenn, 2010; Duek et al., 2001; Eaton and Kells, 2009; Erban et al., 2016; García, 2004; Gill et al., 2011; Hughes, 1976; Kheradmand et al., 2007; Rentfrow et al., 2008; Sánchez-Ramos and Castanera, 2000). Moving mites and piles of pale brownish dust may appear on heavily infested products. The dust piles are consist of waste material, dead mites, and shed cuticle, which have pungent and/or minty odors (Nayak, 2006; Townsend, 2007).

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Some fungi (usually *Penicillium*, *Candida zeylanoides* and *Debaryomyces hansenii*) may be found on the outer surface of the ham (Dikeman and Devine, 2014), which positively influences mite viability and contributes to infestations (Okabe and Oconnor, 2001). Moreover, exposure to *T. putrescentiae* may contribute to allergic diseases, such as contact dermatitis and asthma, among farmers and workers who handle heavily infested stored products (Arlan et al., 2004; Armentia et al., 1994; Fernandez-Caldas et al., 2007; Hage-Hamsten et al., 1985; Revsbech and Dueholm, 1990).

The optimum environmental conditions for mite growth and reproduction include 25 to 32°C and 70 to 90% relative humidity (Boczek, 1991), which are similar to the environmental conditions in dry cured ham aging houses. Mite infestations occur in dry cured ham plants in the southeastern United States (Rentfrow et al., 2008), Spain (Arnau and Guerrero, 1994; Sánchez-Ramos and Castañera, 2001), and other countries, resulting in product rejection and economic losses (Armentia et al., 1994).

In a survey of country ham plants, 22 out of 34 plants reported mite infestations and fumigation with methyl bromide (MB) from 1 to 5 times a year. These plants found that MB is currently the only known space fumigant that is effective at controlling ham mites (Rentfrow et al., 2008). However, since MB is an ozone depleting substance, it was phased out of production and consumption as of January 1, 2005, with the exception of critical use exemptions (EPA, 2017), which were available to the dry cured ham industry through 2016. Beginning in 2017, the dry cured ham industry was only given approval to purchase and use the remaining stockpiles of MB until they are depleted (EPA, 2014). Therefore, it is important to explore alternative control measures to prevent mite infestations during dry cured ham aging since MB will eventually not be possible to use.

Phosphine (Zhao et al., 2015), sulfuryl fluoride (Phillips et al., 2008), controlled atmosphere (Hasan et al., 2016; Sánchez-Molinero et al., 2010), traps (Amoah et al., 2016), high temperature (Abbar et al., 2016b; Fields, 1992), low temperature (Abbar et al., 2016b), and sanitation (Rentfrow et al., 2006) have been evaluated for their efficacy at controlling mite infestations. Coating or rubbing hams during aging with vegetable oils or hot lard is frequently used in Spain to help prevent mite infestations (García, 2004). Researchers determined that lard, propylene glycol, canola oil, calcium sorbate, sodium sorbate, iodate salt, maleic acid, propylol, and butanediol decreased *T. putrescentiae* reproduction when compared to untreated controls (Abbar et al., 2016a; Abbar et al., 2012; Zhao et al., 2016).

Since propylene glycol was the most effective ingredient in the research by Abbar et al. (2012) and Zhao et al. (2016), xanthan gum + propylene glycol and carrageenan + propylene glycol alginate + propylene glycol coatings were developed that were effective at controlling mite reproduction on ham cubes under laboratory conditions (Zhao et al., 2016). Since gum and propylene glycol coatings were applied to the surface of the hams, an additional processing step is necessary. Therefore, research was conducted on the effectiveness of infusing coatings into special nets that are used to hang dry cured hams on racks during aging, which revealed that gum and propylene glycol infused nets effectively inhibited mite growth and reproduction over a 10-wk storage period under laboratory conditions (Zhang et al., 2017). Since lard is used in Europe to control mites and is less costly than propylene glycol, it was hypothesized that incorporating lard into the netting solution, along with propylene glycol could control mites and reduce costs. Therefore, in this study, ham nets were infused with different combinations of polysaccharide coating, lard and propylene glycol. The choice behavior of *T. putrescentiae* between ham cubes wrapped with untreated and treated nets were investigated in addition to the effect of using treated nets on mite reproduction during 10 wk of storage.

## Materials and Methods

### Food ingredients and nets

Xanthan gum (XG; 21 CFR 172.695), carrageenan (CG; 21 CFR 184.1221), and propylene glycol alginate (PGA; 21 CFR 172.858) were provided by TIC Gums, Inc. (Belcamp, MD). Lard (Armour pork fat, 21 CFR 182.70) was purchased from Wal-Mart Stores Inc. (Starkville, MS). Food-grade propylene glycol (PG; 21 CFR 184.1666) was procured from the Essential Depot (Sebring, FL). Ham nets (Ennio International, Aurora, IL) consisted of 50% polyester and 50% cotton (blend nets) with a stitch density of 112 loops/cm<sup>2</sup>.

### Preparation of food-grade ingredients infused nets

The netting solutions consisted of gum (XG or CG + PGA), lard (low or medium concentration), and/or PG (low or medium concentration). The specific concentrations of ingredients cannot be specified because their formulation is intellectual property that is patent pending. To prepare the netting solutions, XG (1%) or CG (1%) + PGA (1%) were mixed with PG followed by mixing

with tap water. The CG + PGA + PG mixtures were then heated to boil while stirring. Lard was melted on a heating plate prior to the addition of the gum and PG solution. The gum, PG, and lard mixtures were blended in a food blender at high speed for 1 min.

Nets were cut, weighed, and then immediately soaked in the newly-prepared netting solution for 10 min. The soaked nets were pressed through 2 rollers of a netting machine (Midwest Metalcraft and Equipment Company, Winsor, MO) to squeeze out the extra fluids. The treated nets were weighed, vacuum packaged into vacuum bags (3 mil. Standard barrier, nylon/PE Clarity Vacuum Pouches; Kansas City, MO) with a dual-chamber vacuum packaging machine (Model 2100, Koch Equipment LL., Kansas City, MO) at full vacuum setting, and kept at room temperature until use. The amount of coating absorbed was  $1.6 \pm 0.3$  g coating/cm of net.

### Ham cubes

Dry cured hams (6 to 8 kg; 4 to 6 mo in age) were purchased from a commercial supplier. The bone-in hams were cut transversally into 1.3 cm and 2.5 cm thick slices. Each slice was vacuum-packaged and stored at 4°C until use. Ham cubes were prepared for the analysis as described below.

### Mite cultures

*T. putrescentiae* stocks were provided by Dr. Phillips's laboratory in the Department of Entomology at Kansas State University and were maintained on a diet as previously reported (Abbar et al., 2016b). The inoculated rearing jar was placed in a locked storage box that contained soapy water at the bottom, and petroleum jelly was smeared on the edges to prevent the mites from escaping. The mite culture was maintained at  $23 \pm 2^\circ\text{C}$  and  $80 \pm 5\%$  relative humidity (RH) in a dark cabinet for 3 to 4 wk prior to use. A polymerase chain reaction cloning method confirmed that the mite culture raised in the laboratory was a pure culture of *T. putrescentiae*.

### Two-choice behavior test of mites

A 2-choice behavior test was conducted according to the methods of Zhang et al. (2017). One 1.3-cm ham cube covered with the control (untreated) net and another 1.3-cm ham cube covered with a treated net were offered simultaneously to the mites inside a small arena. The inside bottom of the plastic Petri dish (150 mm diameter  $\times$  15 mm depth) was covered with black construction paper that was cut to the same dimension as

the Petri dish. Three specific round areas (C, M, and T) were assigned along a line passing through the center of the paper, with M designating the mite release point at the center, C being the ham piece with the untreated net as a control and T as the treated net for a given test, and each ham piece set at 10 mm away from the side wall of the Petri dish. Two ham cubes from the same muscle section were selected and wrapped with control and treated nets, with one control cube placed in area C and one cube placed in area T. Five replications (5 pairs of cubes) were tested for each treatment. Two pairs of cubes were taken from the *biceps femoris* muscle, 1 pair from the *semitendinosus* muscle, and 2 pairs from the *adductor* and *semimembranosus* muscles. A total of 20 mixed sex adult mites (approximately 15 females) were placed in the M area on the paper, and the Petri dish was stored in the dark in a growth chamber ( $23 \pm 2^\circ\text{C}$  and  $80 \pm 5\%$  RH). A thin layer of petroleum jelly was applied on the inner upper 5 mm of the Petri dish to prevent mites from escaping. After 6 h of incubation, mites that were oriented to each of the net-wrapped ham cubes were counted. Orientation was indicated by the number of live mites on the control and treated ham cubes (Abbar et al., 2016a). An identical setup was prepared, and 20 mites were placed in the M area. The number of eggs laid on the cubes and nets were counted after 4 d of incubation. Oviposition was determined by counting eggs that were laid on the control and treated ham cubes.

### Mite reproduction assays

Ham cubes (2.5 cm  $\times$  2.5 cm  $\times$  2.5 cm) were removed from ham muscles and assigned to and packaged with either control or treated nets. Five replications were tested for each treatment. Two cubes were taken from the *biceps femoris* muscle, 1 from the *semitendinosus* muscle, and 2 from the *adductor* and *semimembranosus* muscles. Ham cubes that were packaged in control and treated nets were then placed in ventilated glass Mason jars (216 mL, 65 mm diameter, 55 mm height; Ball Corp., Broomfield, CO). The bottoms of the jars were covered with black construction paper and the tops were covered with filter paper (Whatman Grade 1, Qualitative, Cellulose; GE Healthcare UK Limited, Amersham Place, UK) that was sealed with the jar ring. Three sets of experiments were conducted. The netting formulations in the first set included 100% lard, lard + low-, and medium-PG; the second set included XG + low/medium- lard + low/medium- PG; and the third set included CG + PGA + low/medium- lard + low/medium- PG. Control ham cubes were not covered with nets, while net control ham cubes were

wrapped with untreated nets. Each cube was inoculated with 20 large mixed sex adult mites (predominantly females) and incubated in a dark cabinet that was controlled at  $23 \pm 2^\circ\text{C}$  with a relative humidity of  $80 \pm 5\%$ . To evaluate the long-term effectiveness of treated nets at controlling mite infestations, three batches of samples were prepared and each batch was inoculated with mites either on the first day of storage, after 4 wk of storage, and after 8 wk of storage, respectively. After 2 wk of incubation, the total numbers of moving mites on the ham cubes, nets, black paper, and in the jars were counted under a stereo microscope (Model 568, American Optical Company, Buffalo, NY). The presence of mold and yeast growth on ham cubes were recorded. Molds were characterized by the cottony mycelium growth or colored spores; yeasts were characterized by the growth of slimy, light colored, and defined colonies. The culture of molds or yeasts on ham samples were subcultured on Potato Dextrose Agar plates to confirm their morphological traits.

### **Water activity, moisture content, and weight loss**

Simultaneous experiments were conducted as described above with non-inoculated ham cubes to evaluate water activity ( $a_w$ ), moisture content, and weight loss of the ham cubes from each treatment. Each treatment consisted of 3 replications, 2 ham cubes from the *biceps femoris* muscle, and 1 from the *semitendinosus* muscle. After 4 and 8 wk of storage, water activity of the ham cubes was measured at room temperature with a water activity meter (AquaLab Series 3 TE, Decagon Devices, Inc., Pullman, WA). The moisture content of the samples was determined by drying the minced ham sample ( $2.0 \pm 0.1$  g) in an oven at  $105 \pm 2^\circ\text{C}$  until a constant weight was obtained (AOAC, 2000). The moisture content in percentage was expressed as the difference of the weight before and after drying divided by the initial weight  $\times 100$ . The weights of fresh ham cubes and the weights of ham cubes after 10 wk of storage were taken to calculate the weight loss. Weight loss in percentage was expressed as the difference of the weight before and after the experiment divided by the initial weight (before experiment)  $\times 100$ .

### **Statistical analyses**

Significance of mite preference for a given ham combination (control vs. treated) in the 2-choice behavioral test was calculated by a paired, 2-sided Student's *t* test using Microsoft Excel 2007. A *P*-value  $> 0.05$  was not significant (NS), a *P*-value  $< 0.05$  was considered to

be significant marked with \*, and a *P*-value  $< 0.01$  was marked with \*\*, assuming unequal variances.

A randomized complete block design with 2 replications (batches) and 5 subsamples per replication was used to determine the effect of different treatments on mite reproduction, water activity, moisture content, and weight loss of ham cubes in glass jars. When significant differences ( $P < 0.05$ ) occurred among treatments, Tukey's Honestly Significant Difference Test ( $P < 0.05$ ) was used to separate treatment means.

A  $2^2$  factorial arrangement within a randomized complete block design with 2 replications and 5 subsamples per replication was used to evaluate the effects of using combinations of PG (low- and medium-) and lard (low- and medium-) net treatments on mite reproduction; A  $2^3$  factorial arrangement within a randomized complete block design with 2 replications and 5 subsamples per replication was used to evaluate the effects of combinations of gums (XG, CG + PGA), PG (low- and medium-) and lard (low- and medium-) treatments on water activity, moisture content, and weight loss.

## **Results**

### **Mite behavioral assays**

Mite orientation results indicated that a greater number of *T. putrescentiae* mites were found on the controls than on the treatments of XG + medium lard + medium PG ( $P < 0.01$ ), CG + PGA + low lard + medium PG ( $P < 0.01$ ), or CG + PGA + medium lard + medium PG ( $P < 0.05$ ; Table 1). In addition, there were no differences ( $P > 0.05$ ) between the number of mites on the control and each of the remaining treatments ( $P > 0.05$ ). However, oviposition data indicated a strong egg-laying preference on the control when compared to its treated ham cube pair ( $P < 0.01$ ); on average less than 3 eggs were found on treated ham cubes whereas 74 to 165 eggs were found on untreated ham cubes (Table 1).

### **Mite reproduction assays**

Fewer *T. putrescentiae* mites ( $P < 0.05$ ) were on ham cubes with treated nets containing PG when compared to the number of mites on ham cubes with untreated nets over 10 wk of storage (Tables 2 through 4). In comparison to the net controls (123 to 163 mites on average), lard and low- or medium- PG infused net treatments had 19 to 44 mites (Table 2). However, lard infused nets without PG did not decrease the mite population ( $P > 0.05$ ) (Table 2). The XG + lard + PG infused

**Table 1.** Mean numbers (SD) of mites (orientation) and eggs (oviposition) of *T. putrescentiae* on small dry-cured ham cubes wrapped in untreated control and treated nets in a laboratory 2-choice behavior bioassay ( $n = 5$ )<sup>1</sup>

Treatment <sup>2</sup>	Orientation- mite counts after 6 h			Oviposition- egg counts after 4 d		
	Treated	Control	Difference	Treated	Control	Difference
Lard 100%	4.6 (2.1)	5.4 (3.3)	NS	1.6 (2.3)	84 (26)	**
Lard + low PG	4.2 (2.6)	5.0 (1.6)	NS	0	122 (41)	**
Lard + medium PG	3.4 (2.1)	6.6 (3.0)	NS	0	94 (45)	**
XG + low lard + low PG	4.4 (2.5)	5.2 (3.5)	NS	3.0 (6.7)	69 (27)	**
XG + low lard + medium PG	3.0 (3.3)	6.2 (2.4)	NS	0	80 (18)	**
XG + medium lard + low PG	3.6 (1.9)	3.4 (1.3)	NS	0.6 (1.3)	96 (36)	**
XG + medium lard + medium PG	2.8 (2.5)	8.2 (2.2)	**	0	74 (30)	**
CG + PGA + low lard + low PG	3.0 (2.4)	6.0 (2.1)	NS	1.2 (1.8)	111 (31)	**
CG + PGA + low lard + medium PG	2.6 (1.5)	7.8 (2.6)	**	0	165 (73)	**
CG + PGA + medium lard + low PG	3.4 (2.7)	5.2 (2.9)	NS	0	103 (29)	**
CG + PGA + medium lard + medium PG	1.4 (1.3)	6.8 (3.8)	*	0	97 (41)	**

<sup>1</sup>Pairwise comparison of treated and control orientation and oviposition data followed by a 2-sample Student's *t* test, assuming unequal variances: NS =  $P > 0.05$ , \* =  $P < 0.05$ , \*\* =  $P < 0.01$ .

<sup>2</sup>PG: propylene glycol, XG: xanthan gum, CG: carrageenan, PGA: propylene glycol alginate.

**Table 2.** Mean (SD) population growth of *T. putrescentiae* inoculated on ham cubes that were stored for either 0, 4, or 8 wk after applying the nets with lard and/or propylene glycol infused nets after 2 wk ( $n = 10$ )<sup>1</sup>

Treatment	0-wk-old		4-wk-old		8-wk-old	
	Mites	Fungi	Mites	Fungi	Mites	Fungi
Control	404.8 (155.1)*	M	260.2 (191.9)*	M	193.7 (111.4)	M, Y
Net control	163.1 (65.0) <sup>a</sup>	M	123.1 (39.2) <sup>a</sup>	M	158.0 (126.1) <sup>a</sup>	M
Lard 100%	121.9 (143.0) <sup>ab</sup>	M	81.2 (66.7) <sup>ab</sup>	M	118.1 (78.7) <sup>ab</sup>	M
Lard + low PG	43.9 (34.8) <sup>b</sup>	ND	43.7 (14.8) <sup>bc</sup>	ND	23.2 (38.5) <sup>bc</sup>	M
Lard + medium PG	32.7 (16.3) <sup>c</sup>	ND	18.9 (18.6) <sup>c</sup>	ND	29.1 (36.4) <sup>c</sup>	M

<sup>a-c</sup>Means with same letter within each column are not different ( $P > 0.05$ ) using Tukey's Honestly Significant Difference test.

\*Indicates that control and net control are different ( $P < 0.05$ ).

<sup>1</sup>PG: propylene glycol, M: molds, Y: yeasts, ND: not detected.

nets had fewer mites (2 to 39 mites;  $P < 0.05$ ) when compared to the net control (77 to 146 mites; Table 3). Similarly, CG + PGA + lard + PG infused nets also had fewer mites (0 to 22 mites) ( $P < 0.05$ ) than the net control (88 to 123 mites) over 10 wk of storage (Table 4).

Factorial analysis indicated that the medium PG concentration consistently inhibited mite reproduction ( $P < 0.05$ ) in comparison to treatments without PG that were inoculated with mites at storage periods of 0, 4, and 8 wk after packaging in net treatments (Tables 3 and 4). In addition, the greatest lard concentration decreased mite reproduction in the CG + PGA + lard + PG treatments when mites were inoculated after 4 wk of storage ( $P < 0.05$ ).

Nets slowed the growth and reproduction of *T. putrescentiae* since net controls had fewer mites (77 to 163 mites;  $P < 0.05$ ) than controls without nets (133 to 437 mites). Molds or yeasts were not present on ham cubes that were treated with PG-containing nets over 10 wk of storage, with the exception of lard + PG treat-

ments that were inoculated at 8 wk of storage, and XG + low lard + low PG and CG + PGA + low lard + low PG treatments that were inoculated at 4 wk of storage. This indicates that using the greater concentrations of PG and lard prevents mold and yeast growth, which is an important factor in controlling mite infestations.

### Water activity, moisture content, and weight loss

There were no differences ( $P > 0.05$ ) between control and net control samples in terms of  $a_w$ , moisture content and weight loss (Table 5). The water activity was less ( $P < 0.05$ ) in the net control at 4 wk of storage than the treatments containing gum, lard, and the medium concentration of PG. After 8 wk of storage, the water activity of all samples decreased by 0.02 to 0.08 on average; and the net control had a lower  $a_w$  than all treatments except for the lard + low PG, XG + medium lard + low PG, or CG + PGA + low lard + low PG treat-

**Table 3.** Mean (SD) population growth of *T. putrescentiae* inoculated on ham cubes that were stored for either 0, 4, or 8 wk after applying the nets infused with xanthan gum, lard and propylene glycol after 2 wk ( $n = 10$ )<sup>1</sup>

Treatment	0-wk-old		4-wk-old		8-wk-old	
	Mites	Fungi	Mites	Fungi	Mites	Fungi
Control	436.6 (132.4)*	M	132.7 (118.1)*	M, Y	157.9 (134.7)	M, Y
Net control	146.2 (83.7) <sup>a</sup>	ND	76.6 (57.2) <sup>a</sup>	M, Y	126.5 (147.6) <sup>a</sup>	Y
XG + low lard + low PG	38.6 (32.7) <sup>b</sup>	ND	28.6 (14.0) <sup>b</sup>	M	17.5 (17.9) <sup>b</sup>	ND
XG + low lard + medium PG	20.9 (18.8) <sup>b</sup>	ND	4.6 (4.6) <sup>b</sup>	ND	3.1 (2.4) <sup>b</sup>	ND
XG + medium lard + low PG	25.3 (24.6) <sup>b</sup>	ND	17.9 (15.0) <sup>b</sup>	ND	11.2 (13.2) <sup>b</sup>	ND
XG + medium lard + medium PG	12.5 (10.2) <sup>b</sup>	ND	1.9 (1.7) <sup>b</sup>	ND	2.0 (2.1) <sup>b</sup>	ND

<sup>a,b</sup>Means with same letter within each column are not different ( $P > 0.05$ ) using Tukey's Honestly Significant Difference test.

\*Indicates that control and net control are different ( $P < 0.05$ ).

<sup>1</sup>XG: xanthan gum, PG: propylene glycol, M: molds, Y: yeasts, ND: not detected.

**Table 4.** Mean (SD) population growth of *T. putrescentiae* inoculated on ham cubes that were stored for either 0, 4, or 8 wk after applying the nets infused with carrageenan, propylene glycol alginate, lard, and propylene glycol after 2 wk ( $n = 10$ )<sup>1</sup>

Treatment	0-wk-old		4-wk-old		8-wk-old	
	Mites	Fungi	Mites	Fungi	Mites	Fungi
Control	415.0 (155.2)*	M	277.1 (203.5)*	M, Y	88.8 (51.6)	M, Y
Net control	123.1 (74.0) <sup>a</sup>	M	88.1 (55.5) <sup>a</sup>	M, Y	108.5 (81.0) <sup>a</sup>	M, Y
CG + PGA + low lard + low PG	21.7 (16.4) <sup>b</sup>	ND	13.9 (14.0) <sup>b</sup>	M	7.8 (4.7) <sup>b</sup>	ND
CG + PGA + low lard + medium PG	1.7 (1.6) <sup>b</sup>	ND	0.5 (1.0) <sup>b</sup>	ND	0.9 (1.4) <sup>b</sup>	ND
CG + PGA + medium lard + low PG	9.6 (7.9) <sup>b</sup>	ND	1.4 (2.8) <sup>b</sup>	ND	4.9 (9.9) <sup>b</sup>	ND
CG + PGA + medium lard + medium PG	3.6 (3.4) <sup>b</sup>	ND	0.4 (0.7) <sup>b</sup>	ND	0.4 (0.7) <sup>b</sup>	ND

<sup>a,b</sup>Means with same letter within each column are not different ( $P > 0.05$ ) using Tukey's Honestly Significant Difference test.

\*Indicates that control and net control are different ( $P < 0.05$ ).

<sup>1</sup>CG: carrageenan, PGA: propylene glycol alginate, PG: propylene glycol, M: molds, Y: yeasts, ND: not detected.

**Table 5.** Water activity ( $a_w$ ) and moisture content of dry cured ham cubes wrapped with different treated nets after 4 and 8 wk of storage and weight loss of dry cured ham cubes after 10 wk of storage at  $23 \pm 2^\circ\text{C}$  and relatively humidity of  $80 \pm 5\%$ <sup>1</sup>

Treatments	Water activity		Moisture content %		Weight loss %
	4-wk	8-wk	4-wk	8-wk	10-wk
Net control	0.84 (0.02) <sup>c</sup>	0.76 (0.03) <sup>cd</sup>	43.0 (6.5) <sup>b</sup>	36.8 (2.8) <sup>b</sup>	58.8 (1.0) <sup>a</sup>
Lard 100%	0.86 (0.02) <sup>bc</sup>	0.81 (0.02) <sup>b</sup>	48.3 (5.1) <sup>ab</sup>	41.8 (1.5) <sup>ab</sup>	51.8 (2.9) <sup>bc</sup>
Lard + low PG	0.85 (0.02) <sup>bc</sup>	0.79 (0.02) <sup>bc</sup>	47.0 (7.7) <sup>ab</sup>	40.1 (3.9) <sup>ab</sup>	53.5 (3.7) <sup>b</sup>
Lard + medium PG	0.85 (0.02) <sup>bc</sup>	0.82 (0.05) <sup>ab</sup>	47.2 (4.3) <sup>ab</sup>	43.1 (6.2) <sup>ab</sup>	48.6 (1.7) <sup>bcde</sup>
XG + low lard + low PG	0.87 (0.01) <sup>abc</sup>	0.82 (0.03) <sup>ab</sup>	47.8 (4.1) <sup>ab</sup>	43.8 (5.6) <sup>ab</sup>	52.2 (4.1) <sup>bc</sup>
XG + low lard + medium PG	0.88 (0.02) <sup>ab</sup>	0.84 (0.03) <sup>ab</sup>	50.1 (2.3) <sup>a</sup>	45.2 (5.3) <sup>ab</sup>	50.9 (2.3) <sup>bc</sup>
XG + medium lard + low PG	0.86 (0.02) <sup>bc</sup>	0.80 (0.02) <sup>bc</sup>	47.7 (5.0) <sup>ab</sup>	39.3 (3.5) <sup>ab</sup>	51.9 (1.4) <sup>bc</sup>
XG + medium lard + medium PG	0.88 (0.01) <sup>ab</sup>	0.86 (0.02) <sup>a</sup>	49.1 (3.1) <sup>ab</sup>	47.0 (4.7) <sup>a</sup>	45.4 (3.3) <sup>de</sup>
CG + PGA + low lard + low PG	0.84 (0.01) <sup>c</sup>	0.80 (0.02) <sup>bc</sup>	47.6 (2.1) <sup>ab</sup>	43.5 (8.2) <sup>ab</sup>	50.0 (1.3) <sup>bcd</sup>
CG + PGA + low lard + medium PG	0.87 (0.02) <sup>ab</sup>	0.82 (0.01) <sup>ab</sup>	48.4 (2.4) <sup>ab</sup>	47.8 (8.1) <sup>a</sup>	47.1 (1.1) <sup>cde</sup>
CG + PGA + medium lard + low PG	0.87 (0.01) <sup>abc</sup>	0.82 (0.01) <sup>ab</sup>	50.5 (3.3) <sup>a</sup>	41.7 (1.9) <sup>ab</sup>	49.1 (4.5) <sup>bcde</sup>
CG + PGA + medium lard + medium PG	0.90 (0.01) <sup>a</sup>	0.86 (0.02) <sup>a</sup>	51.3 (1.9) <sup>a</sup>	46.2 (1.9) <sup>ab</sup>	44.0 (0.9) <sup>e</sup>
SEM	0.002	0.003	0.400	0.584	0.295

<sup>a-c</sup>Means with same letter within each column are not different ( $P > 0.05$ ) using Tukey's Honestly Significant Difference test.

<sup>1</sup>PG: propylene glycol, XG: xanthan gum, CG: carrageenan, PGA: propylene glycol alginate.

ments. Moisture content decreased as ham cubes were stored in the humidity controlled cabinet (Table 5). The net control contained the least moisture, which was less than CG + PGA + medium lard + low/medium PG and XG + low lard + medium PG after 4 wk of storage, and less than XG + medium lard + medium PG and CG + PGA + low lard + medium PG treatments after 8 wk of storage. After 10 wk of storage, all treatments had less weight loss ( $P < 0.05$ ) than the net control with CG + PGA + medium lard + medium PG being the lowest (44%). Addition of PG to the net coating treatments led to increased moisture content and decreased weight loss ( $P < 0.05$ ). In addition to the net coating treatments, medium PG treatments retained more moisture than the low PG treatments. However, coating nets with treatments did not prevent moisture loss from the hams.

## Discussion

The purpose of this study was to develop nets in which lard was infused with food-grade coatings and evaluate their effectiveness at inhibiting mite contact and reproduction. The net used in this study was a blend of 50% polyester and 50% cotton with high stitch density (112 loops/cm<sup>2</sup>). The nets without a coating decreased the mite population since the nets reduced the meat exposure to mites and limited their actual locomotion, which is in agreement with the previous findings (Campbell et al., 2016; Zhang et al., 2017). There are a few commercially available mite-proof covers that are made from different materials, including a tightly woven cover with a pore size of 2 to 10  $\mu\text{m}$  (Mahakittikun et al., 2006; 2009), cotton fabric coated with eugenol loaded chitosan (Jarupaiboon et al., 2007), and Chitosan/Ag<sup>+</sup>-impregnated textile (Rahel et al., 2013). These anti-mite covers prevent the settlement and development of house dust mites, *Dermatophagoides farinae* Hughes on household fabrics and other surfaces, but have not been tested for ham mites. However, the usability of a tightly woven cover in the ham industry needs to be evaluated because of its low air permeability (4.0 cm<sup>3</sup>/s/cm<sup>2</sup>). In addition, neither of the 2 fabrics that are used for protection from house dust mites can be applied in the ham industry due to the cost and the use of non-food-grade materials. The coated nets used in the ham industry should contain only food-grade (Generally Recognized As Safe, GRAS) ingredients that are approved for food contact (FDA 21 CFR; Baldwin et al., 2011), which are gum, propylene glycol and lard in this study, and have no side effect on ham aging process and ham quality.

Data from behavior assays indicated that *T. putrescentiae* avoided ham cubes with some of the treated nets, and they showed strong preference for oviposition on control ham cubes over any of the treated ham cubes. The reproduction test suggested that nets containing PG reduced the mite population and the inhibitory effect was maintained over the 10-wk storage period, which is in agreement with previous findings on research conducted on gums and PG infused nets (Zhang et al., 2017). The main differences between these 2 studies were the incorporation of lard into the netting formulation and the infusion of nets with different combinations of gum, lard, and PG, which was due to the fact that coating ham cubes with lard was effective at inhibiting *T. putrescentiae* reproduction and has been used in commercial practice (Abbar et al., 2016a; Zhao et al., 2016).

The 100% lard infused nets were avoided by *T. putrescentiae* for oviposition but did not decrease the mite population when compared to the net control. Since 2 ham cubes with untreated and lard treated nets were provided, mites likely chose to lay eggs on control ham cubes because they were preferred by the mites. However, ham cubes with lard infused in the nets could sustain mite growth if mites had no other options for growth. For example, in the reproduction studies, ham cubes that were covered with lard infused net was the only food source to mites. When lard coating was used instead of lard infused nets, the mite population was controlled in the reproduction assay (Zhao et al., 2016), which might be due to the fact that the lard coating provided a lipid barrier that blocked the access of mites to feed on the meat. In contrast, the mesh size of the nets that were used was not small enough to prevent mites from penetrating through the nets to the ham meat.

Propylene glycol is the key ingredient that inhibits mite infestations on dry cured ham (Abbar et al., 2016a; Zhao et al., 2016). Propylene glycol is a clear, viscous, and colorless liquid with minimal odor and a slightly bittersweet taste (Martin and Murphy, 2000). Propylene glycol is classified as a GRAS ingredient by the FDA when used as a direct additive in food, cosmetic, and pharmaceutical products (Anonymous, 1994; Fiume et al., 2012). When compared to the net control, the lard + low/medium PG infused nets reduced the mite population by 73.1/80.0%, 64.5/84.6%, and 85.3/81.6% for treatments that were immediately inoculated after netting, 4 wk after netting and 8 wk after netting ham samples inoculated, respectively. This confirms the effectiveness of PG at inhibiting mite reproduction.

For the combinations of gum, PG and lard, all treated nets reduced the number of mites as compared to the net control over 10 wk of storage, with reductions

of the mite population by 63 to 99%. The inhibitory effect on mite reproduction was dependent on the inclusion of PG and lard. In general, as PG increased, the mite population decreased. Similarly, as lard increased, the mite population also decreased, but the rate of decrease was less than when PG was increased. When the same combination of lard and PG was used, CG + PGA treatments had fewer mites than XG treatments. Although there were no statistical difference among the four different treatments at each storage time point, CG + PGA + medium lard + medium PG had the fewest mites present and would be preferred for use because of the zero tolerance of mites on dry cured ham products in the United States (USDA 9 CFR301; USDA 9 CFR 416). When compared to previous research from our laboratory with XG / (CG + PGA) + medium PG infused nets (Zhang et al., 2017), the nets containing the same concentration of PG did not improve the inhibitory effect, indicating that adding lard to the formulation did not offer any added benefit.

Another important property of PG is that it helps control the water activity and thereby spoilage in intermediate moisture or semi-moist foods (Aldrich, 2014), which may explain no or less growth of fungi on the ham cubes that were covered with PG-containing nets. Nets containing PG inhibited fungal growth with only a few exceptions, including lard + PG treatments after 8 wk of storage and XG / (CG + PGA) + low lard + low PG treatments after 4 wk of storage. In comparison, fungal growth was present on ham cubes without nets and with untreated nets as well as with 100% lard treatments. Mold growth usually favored the reproduction of *T. putrescentiae*, which confirms that the *T. putrescentiae* mite is fungivorous (Okabe and Oconnor, 2001). Molds might serve as the food source of mites (Hubert et al., 2004), or a source of free water and shelter for mites (Canfield and Wrenn, 2010). In contrast to the moldy ham cubes, the samples with visible yeasts had a pungent smell that was obvious to the investigators and fewer mites than the same treatment without yeast. The typical mold and yeast colonies were isolated and saved for future species identification, which will be conducted by another student in our laboratory.

Dry cured ham develops the typical flavor through the breakdown of proteins and fatty acids into volatile compounds (Pham et al., 2008). In addition, dry cured hams lose moisture by water evaporation and air exchange during the aging, and the finished dry cured ham product has to lose at least 18% of its original weight (Marriott and Ockerman, 2004). Therefore, any coatings, including coated nets must be permeable to moisture so that the hams can lose more than

18% moisture and become shelf-stable. Nets containing lard, PG, or both retained more moisture than other treatments. Therefore, the ham cubes with these nets had higher water activity and lower weight loss than ham cubes with control nets after 4 and 8 wk of storage. This is due to the nature of lard and PG. Lard is strictly hydrophobic and presents a barrier to water vapor. Propylene glycol is a humectant. It is miscible with water and can easily bind water from the atmosphere. The samples used for moisture analysis were small ham cubes, weighing approximately 20 g. Whole hams that are aged for 4 to 6 mo usually weigh 5 to 7 kg, 300 times heavier than the small cubes. Therefore, the water activity, moisture, and weight loss difference between control and treated samples would be much less in commercial products. However, even though the control treatments had less moisture and a lower  $a_w$ , ham cubes were still able to lose moisture and have an acceptable  $a_w$ , which was 0.76 after 8 wk of storage. Therefore, it is suggested that these nets could be used in commercial production to inhibit mite infestations since hams could still undergo moisture loss and the aging process.

The nets infused with CG + PGA + medium lard + medium PG were the most effective at inhibiting mite growth and reproduction, indicating that the combination of these ingredients was effective at controlling mite infestation and fungal growth. However, there were still mites alive after this treated net was applied, which did not meet the requirement of zero tolerance, even though there were fewer mites than the initial inoculation level of 20. It is recommended that mite infestations are controlled using an integrated pest management (IPM) program, with the use of coated nets as part of the program. In the IPM program, prevention should always take place at the earliest time of processing. For example, if the ham is infested, cleaning dry cured ham with vegetable oil, as mentioned earlier, only eliminates the mites on the outer layer but not mites in cracks; it would be much complicated to excavate mites inside the cracks. Thus, multiple hurdles need to be considered part of IPM program, including monitoring, sanitation, prompt action to possible infestations, fumigation, etc.

## Conclusions

Gum and medium PG treated nets were effective at controlling mites during aging. This technique would be a helpful addition to an IPM program to control ham mites and can be considered a potential alternative to MB fumigation if and when MB fumigation is no longer allowed.



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