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# Use of nets treated with food grade coatings on controlling mold growth and mite infestation in dry-cured ham aging facilities

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ABSTRACT

Dry-cured hams are frequently infested by mold and *Tyrophagus putrescentiae* in the aging facility during the 3 mo to 2-year aging period. Food grade coatings and these coating treated nets have been developed to control mold and mite growth on dry-cured hams to curtail the use of methyl bromide. Ham nets treated with food-grade coating of 1% propylene glycol alginate + 1% carrageenan + 40% propylene glycol were tested in a commercial research trial in 3 lots with approximately 100 hams in each lot, which also had approximately 100 control hams (untreated). Six of these hams were sent back to the research team. Three of these hams (already aged for 8 mo) were kept in a mite-infested simulated aging room for further evaluation of mold occurrence and mite population growth for another 6 mo. The other 3 hams from each lot of each treatment were tested by gas chromatography for propylene glycol residual. Mold evaluation indicated that the treatment hams reduced mold occurrence compared to the control hams, and there were no mite activities in any of these hams per inspection. In the mite-infested aging room, these coating-treated nets reduced mold and mite growth on whole hams (8–14-month-old). There was no difference in propylene glycol concentration between the control (0.072%) and net treated samples (0.053%). This concentration is 4 times less than 2%, the maximum acceptable concentration of this GRAS compound based on CFR 21,184.1666. Therefore, these hams met the legal requirements for commerce and were safe for human consumption and can be used to mitigate and help control mite infestations and mold growth of hams that are aged longer than 5 months.



## 1. Introduction

Dry-cured hams, also known as “Country Hams”, or “Country Style Ham” in the United States, are uncooked, cured, dried and smoked or unsmoked pork products that are made from the hind leg of the hog (USDA FSIS, 9 CFR 319.106, 2020). Dry-cured hams are cured with salt, or salt combined with any of the following ingredients: nitrite or nitrate, nutritive sweeteners, spices, seasonings, and flavorings (USDA FSIS, 9 CFR 319.106, 2020) for 2 weeks. Per definition, dry-cured hams must contain a minimum of 4% salt with a water activity of or below 0.92. During aging, the hams are placed in the aging houses with a temperature range of 16–25 °C for Europe and greater than 28 °C for the United States and a relative humidity range of 65–85% for 3 mo and up to 24 mo (Toldrá and Aristoy, 2010; Rentfrow et al., 2012).

The ham mite, Tyrophagus putrescentiae (Schrank), also known as the cheese mite or mold mite, is the predominant pest found in dry-cured ham aging houses. Ham mites (T. putrescentiae) can produce allergens for human beings, as well as their feces (Arlian et al., 1984). T. putrescentiae is considered as the third most common house mite, one of the most common cause of allergic sensitization in respiratory allergic patients around the globe (Munhbayarlah et al., 1998). Therefore, controlling mite growth is crucial in reducing the health risk of storage product plant workers and consumers (Hubert et al., 2003, 2004). Dry-cured hams have a high susceptibility to mite infestations, starting at 4–6 mo into the aging process, due to their high fat and protein content, water activity, and moldy surface, (García, 2004; Rentfrow et al., 2008). The optimal growth conditions for ham mites are 23.2 ± 2.1 °C and 71 ± 5.6% relative humidity (Sánchez-Ramos and Castañera, 2005;

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Aspaly et al., 2007; Sánchez-Ramos et al., 2007; Qu et al., 2015), which is similar to the environment in dry-cured ham aging houses (Sánchez-Ramos and Castañera, 2000; Rentfrow et al., 2012). These environmental conditions with the lack of lighting in the aging houses foster and promote the growth of both mold and T. putrescentiae, and the mold provides food and water for the mites (Canfield and Wrenn, 2010).

Methyl bromide fumigation has been the standard method to control ham mite infestations due to its effectiveness in aging facilities for decades (Fields and White, 2002; Zhao et al., 2016a). With the phase-out of methyl bromide, the dry-cured ham industry needs effective alternatives to control mite infestations in the aging rooms. Food grade coatings with propylene glycol (PG; active ingredient) and polysaccharides have been developed to control mite growth on dry-cured ham cubes (Zhao et al., 2016b). Further research demonstrated that dipping or spraying whole hams with these coatings showed minimal impact on the sensory qualities on whole hams in the aging houses from different facilities (Campbell et al., 2017). Treating textiles to control pests has been previously evaluated. Rahel et al., 2012) treated polypropylene, non-woven textile with chitosan and metal ions (Cu2+, Ag+, Zn2+). These authors reported that plasma treated fibers with chitosan and Ag + controlled the reproduction of T. putrescentiae and other synanthropic mites. These textiles were treated with heavy metal ions and/or non-food ingredients and cannot be used on dry-cured hams. Treating cotton or polyester nets with these coatings (1% PGA + 1% CG + 40% PG) was also effective at controlling mite growth on dry-cured ham cubes (Campbell et al., 2018). Zhang et al. (2018) also demonstrated that these coating-treated nets were able to control mite growth for up to 8 weeks in laboratory settings. The studies listed above were done in laboratory settings. Therefore, in-plant commercial testing was needed to test the effectiveness of these coating-treated nets on whole hams.

According to the Code of Federal Regulation and USDA FSIS, meat products may contain up to 2% PG in the product (21 CFR 186.1666, USDA FSIS Directive). PG is considered a generally recognized as safe (GRAS) substance by the FDA (FDA., 2015). However, this substance can be a potential health problem when consumed more than the recommended amount (25 mg/kg/day) (Fowles et al., 2013; Mandl, 2018). Not many cases of PG related toxicity have been reported since this substance is easily be metabolized and removed from a healthy human body (Mandl, 2018). However, an increased risk exists in people with liver or kidney disease (Mandl, 2018; Zar et al., 2007). One method that has been widely used and proven effective to measure PG residual level is the use of gas chromatography to detect in human blood, plasma, or urine (Wurita et al., 2013; Meyer et al., 2011). Propylene glycol is required to be at a minimum of 7.5% for carrageenan and propylene glycol alginate coating and 15% for xanthan gum coating in the study of Campbell et al. (2017). According to Castle et al. (1988), PG can migrate into food, which in this case into hams through contact and adulterate the dry-cured hams. Therefore, research was conducted to 1) evaluate the efficacy of the coated ham nets to control mold and mites in a commercial facility; 2) evaluate the efficacy of the treated ham nets to control mold and mites in a mite-infested simulated aging room; and 3) determine if propylene glycol migrated from the treated nets into the ham during aging.

## 2. Materials and methods

### 2.1. Food-grade coating manufacturing

Propylene glycol alginate (PGA) and carrageenan (CG) were purchased from TIC Gums (White Marshal, MD). Food-grade propylene glycol (PG) was supplied by Hawkins, Inc (Minneapolis, MN) and polyester nets were supplied by Dickson Industries (Des Moines, Iowa). The PGA (1%, 100 g) and CG (1%, 100 g) polysaccharides were added to the propylene glycol (40%, 4000 mL) solution, then the mixture was added to warm water (58%, 5800 mL) slowly. The 10-L mixed solution was then heated to a minimum of 85 °C to ensure that the polysaccharides were completely solubilized.

### 2.2. Treated ham nets manufacturing

Polyester nets (152 loops/cm$^2$) were soaked in the coatings (1% PGA + 1% CG+ 40% PG + 58% water) for approximately 1 min and then fed through a netting machine with an automated double roller system (Midwest Metal Craft & Equipment, Winsor, MO) with heating modifications. The 10 L batches of coating solutions were heated to a minimum of 85 °C and a maximum of 100 °C, then cooled down with ice water bath to 76 °C. The coating solution was poured into the heating tank of the netting machine, and the coating solution temperature was maintained between 55 and 65 °C. The roll of nets was fed through a double roller slowly to ensure that the solution absorbance was maintained at a target of 325–350 g/m (averaged 342.4 g/m). The nets were then rolled back, and vacuum (dual chamber ULTRAVAC, Model UV2100, Koch Equipment, Kansas City, MO) packaged at a vacuum level of 99% into vacuum bags (standard barrier, PVdC, 36 cm × 51 cm, WVTR≈ 0.4 g/100 in2/24 h, Curwood, Inc., New London, WI). These food-grade coating treated nets were produced in the food processing pilot plant at Mississippi State University for the commercial trial. The nets were stored in the facility at room temperature.

### 2.3. Manufacturing of the and hams and netting the hams

These coating treated nets were used in a commercial aging house in the Southeastern United States. Hams were produced using the company's current processing procedures. Hams were salted twice, with 5–6 days in between the first salt and second salt. Hams were washed 30 days after the 2nd salt, for a total of 35–36 days in salt. Hams were stored at 2–4 oC during salting. Hams were then placed in a drying room for 14 days at 8.4 °C and 68.4% relative humidity (RH). After drying, three lots of hams were hung in the commercial aging facility. Nets were cut to 75–90 cm to fit the hams and the end of the net was tied in a knot to secure and completely cover the surface of the hams. One hundred hams were hung in control nets in each of the chosen three lots, and 100 hams were hung in treated nets in each of these three lots, for a total of 300 control hams and 300 treated hams in the study. Hams were coated with nets after drying, with one lot placed in each aging room. Lot A was stored in Aging room 1 (28.2 °C, 62.1% RH), Lot B was stored in aging room 2 (28.8 °C, 68.8% RH), and Lot C was stored in aging room 3 (28.3 °C, 61.3% RH) for Lot C.

### 2.4. Mold and mite growth evaluation in a commercial aging facility

Ten control hams and 10 treated hams were randomly selected and observed weekly from each of 3 lots for 90 days. Mold was evaluated using a 0 to 100 percentage scale, where 0% had no visible mold and 100% indicated that visible mold covered the entire lean portion of the ham. Mite activity was inspected with naked eyes. After the results were obtained for 90 days of aging, the hams continued to age at the commercial facility. After day 90, further mold and mite growth was inspected as needed per the manufacture's practice. Three samples from each house were collected from the aging houses after approximately 8 mo of aging to determine PG concentration in the hams. After 90–600 days of

aging, the nets were removed and disposed of before packaging for commerce.

### 2.5. Mold and mite growth evaluation in the infested simulated aging room

Six control hams and 6 treated hams from each lot were sent back to the research team after approximately 8 mo of aging at the commercial aging. Three hams from each treatment for each lot were placed in an infested simulated aging room at Mississippi State University Enology Lab for further monitoring. The storage room (27 $m^3$) that was used for continuous aging was heavily infested with mites from experiments by Campbell et al. (2017), with approximately 16,000 mites released on to 16 hams (1000 mites each) in 2016. These mites are from the same culture that has been used in previous studies (Abbar et al. 2016; Zhao et al. 2016b; Campbell et al., 2018; Zhang et al., 2018; Hendrix et al., 2018). Hams were monitored for 6 mo in complete darkness at 23 ± 2 °C and 70% ± 5% RH, during the 9–15 months aging period. Mold evaluation was done as described above. Mite evaluation was estimated in the dark by closely inspecting all the surfaces of the hams with a Utilitech 20-lumen LED Rechargeable miniature flashlight (LG Sourcing, Inc., Wilkesboro, NC) and estimating all mobile mites that were seen.

### 2.6. Propylene glycol residual detection using gas chromatography

Muscle samples (2.5 × 2.5 × 2.5 $cm^3$ cubes) were taken from the lean surface of the ham (cushion), the middle point of the ham (about 4.5 cm between the lean surface and the center of the ham), and the center of the ham (near the bone, about 9 cm to the cushion surface). Samples were hand-minced and then homogenized in liquid nitrogen to a fine powder. One hundred mg of sample was weighed into a polypropylene micro-centrifugal tube with deuterated PG (d8; 1000 μg/mL) and acetonitrile (0.75 mL). Propylene glycol was extracted through vigorous mixing and sonication. The extract was centrifuged, and the supernatant was transferred to a GC amber vial to remove the solvent with a gentle stream of nitrogen gas. The residue was derivatized by heptafluorobutyric anhydride in acetonitrile. The derivatives of propylene glycol and propylene glycol d8 were extracted in hexane, which was dried by anhydrous sodium sulfate prior to injection into a GC-MS system. The GC system was equipped with a DB-5 column (30 m, 0.25 mm i.d., 0.25 μm film thickness). The injector temperature was operated in splitless mode at 300 °C with He as a carrier gas. An Agilent 5975C inert XL MSD with a triple-axis mass detector was used with temperature settings of 200 °C and 150 °C for the ion source and quadrupole, respectively. Selected ion monitoring of m/z 241 for propylene glycol and m/z 245 for propylene glycol-d8 was used. Propylene glycol concentration was expressed as percentage of the samples.

### 2.7. Statistical analysis

A 2 (treatment) × 12 (week) factorial arrangement in a randomized complete block design with 3 replications (lots) and 10 subsamples per replication was utilized in the commercial research trial for the mold evaluation. A 2 (treatment) × 7 (week) factorial arrangement of a randomized complete block design with 3 replications (lot) and 3 subsamples (ham) per replication was utilized for the experiment in the simulated aging room for evaluating mite and mold growth on the sampled whole hams in the basement of Mississippi State University Enology Lab. A 2 (treatment) × 3 (ham location) factorial arrangement of a randomized complete block design with 3 replications (ham) and 3 subsamples (location) per replication was used for the PG residual detection analysis by gas chromatography. When significance differences ($P < 0.05$) occurred among treatments, Tukey's Honestly Significant Difference Test ($P < 0.05$) was used to separate treatment means.

## 3. Results

### 3.1. Mold growth on whole hams from the commercial aging facility

There was less mold on whole hams with treated nets in comparison to hams with control nets for lot B ($F_{1, 216} = 238.4$, $P < 0.001$) (Table 1).There was no mold on hams in lot A and C over the 90-day evaluation period) (Table 1). The overall mold growth on whole hams over the 3 lots was reduced in the treated nets in comparison to the hams with control nets ($F_{1, 694} = 72.21$, $P < 0.001$) (Table 1). There was no mold growth in Lot A and Lot C for both control hams and treatment hams (Table 2). In Lot B on the treatment hams, there was no mold growth; on the control hams, the mold ranged from 0% to 18% from week 1 to week 12. Mold growth was less on whole hams with treated nets in all weeks except week 6, 7 and 11 in lot B (F11, 216 = 16.53, $P < 0.0001$) and the overall of all three lots ($F_{11, 694} = 4.96$, $P < 0.0001$) (Table 2).

During the 90 d of monitoring, there was no mite detected with the naked eye. After approximately 14 mo of aging, "there were no mites nor significant mold on test hams, and no mites and minimal mold on control hams" (Edwards et al., 2019).

### 3.2. Mold growth on whole hams in a mite-infested simulated aging room

There was less mold on whole hams with treated nets in comparison hams with control nets for hams from all the individual lots (A: $F_{1, 40} = 99.0$, $P < 0.0001$; B: $F_{1, 40} = 65.8$, $P < 0.0001$; C: $F_{1, 40} = 32.1$, $P < 0.0001$) and the overall ($F_{1, 148} = 112.5$, $P < 0.0001$) (Table 3). There were 16.9%–32.1% mold on the control hams surfaces, while the treatment hams had significantly lower percentage of surface mold ranging from 1.2 to 6.2% (Table 3). Throughout each week, the surface mold was more on the control hams than the treated hams overall for the three lots ($F_{6,148} = 3.7$, $P < 0.0001$), lot A (except week 1), lot B (except week 10 and 25) and lot C (except week 1, 5, 12 and 13) (Table 4). These hams were from the 3 lots (3 hams each) and were stored in the same room. Overall, the control hams had 11.8 (week 1) to 33.6% (week 25) and the treatment hams had 0 (week 1) to 7.2% (week 10) surface mold (Table 4).

### 3.3. Mite growth on whole hams in a mite-infested simulated aging room

There was no mite growth reported in the commercial trial data (per inspection and owner's communication). After these hams were placed in the infested room at Mississippi State University, the control hams had more mites (1136) than the treatment hams (197)

**Table 1**
Mold percentage on research trial hams (n = 10, rep = 3 lots) with control and hams treated with coating for 12 weeks in a commercial aging facility.

| Treatment | Surface Mold (%) | | | |
|---|---|---|---|---|
| | Lot A | Lot B | Lot C | Overall |
| Control | 0 | 13.2 ± 1.3a | 0 | 4.4 ± 0.6a |
| Treatment | 0 | 0 ± 0b | 0 | 0 ± 0b |

Mean ± SE with the same letter within each column are not different (Tukey HSD at P = 0.05).
Control = hams without being treated; Treatment = hams treated with PGA + CG + 40% PG coating.

**Table 2**
Mold percentage on trial hams with control and hams treated with for 12 weeks in a commercial aging facility, within each lot and each week (rep = 3, n = 10). Mean ± SE with the same letter within each column are not different (Tukey HSD at P = 0.05).

| ID | Week | Surface mold (%) | | | |
|---|---|---|---|---|---|
| | | Lot A | Lot B | Lot C | Overall |
| Control | 1 | 0 | 18.0 ± 2.5c | 0 | 6.2 ± 1.8bc |
| Treatment | 1 | 0 | 0e | 0 | 0d |
| Control | 2 | 0 | 17.0 ± 2.0c | 0 | 5.7 ± 1.6bc |
| Treatment | 2 | 0 | 0e | 0 | 0d |
| Control | 3 | 0 | 13.5 ± 1.3d | 0 | 4.5 ± 1.3c |
| Treatment | 3 | 0 | 0e | 0 | 0d |
| Control | 4 | 0 | 25.0 ± 1.5b | 0 | 8.3 ± 2.2b |
| Treatment | 4 | 0 | 0e | 0 | 0d |
| Control | 5 | 0 | 10.6 ± 5.2d | 0 | 3.0 ± 1.8cd |
| Treatment | 5 | 0 | 0e | 0 | 0d |
| Control | 6 | 0 | 0e | 0 | 0d |
| Treatment | 6 | 0 | 0e | 0 | 0d |
| Control | 7 | 0 | 0e | 0 | 0d |
| Treatment | 8 | 0 | 0e | 0 | 0d |
| Control | 8 | 0 | 40.0 ± 2.6a | 0 | 13.3 ± 3.6a |
| Treatment | 8 | 0 | 0e | 0 | 0d |
| Control | 9 | 0 | 18.0 ± 5.7c | 0 | 6.0 ± 2.4b |
| Treatment | 9 | 0 | 0e | 0 | 0d |
| Control | 10 | 0 | 2.0 ± 1.3e | 0 | 0.7 ± 0.46d |
| Treatment | 10 | 0 | 0e | 0 | 0d |
| Control | 11 | 0 | 0e | 0 | 0d |
| Treatment | 11 | 0 | 0e | 0 | 0d |
| Control | 12 | 0 | 16.0 ± 4.5cd | 0 | 5.3 ± 2.0bc |
| Treatment | 12 | 0 | 0e | 0 | 0d |

Control = hams without being treated; Treatment = hams treated with PGA + CG + 40% PG coating.

**Table 3**
Mold growth estimate by lot on trial hams placed in an infested room with mites from previous whole ham experiments (more than 10,000 mites released in the room in 2016, at 20–25 °C, RH 60–80%) after aging approximately 9 m at the commercial aging house (n = 3).

| ID | Surface mold (%) | | | |
|---|---|---|---|---|
| | Lot A | Lot B | Lot C | Overall |
| Control | 25.6 ± 3.1a | 32.1 ± 2.9a | 16.9 ± 3.8a | 24.6 ± 2.0a |
| Treatment | 1.2 ± 0.4b | 6.2 ± 1.4b | 3.7 ± 1.5b | 3.7 ± 0.7b |

Mean ± SE with the same letter within each column are not different (Tukey HSD at P = 0.05).
Control = hams without being treated; Treatment = hams treated with PGA + CG + 40% PG coating.

overall ($F_{1,148}$ = 361.8, $P$ < 0.0001) (Table 5). The same trend occurred for hams from each lot (A: $F_{1, 40}$ = 312.7, $P$ < 0.0001; B: $F_{1, 40}$ = $P$ < 0.0001; C $F_{1, 40}$ = $P$ < 0.0001). No mite activity could be detected by the naked eye on the nets until week 11. From week 12 to week 25, the control hams had more mite growth than the hams with coating treated nets each week within each lot and overall lots except lot C on week 12 and 13 ($F_{6, 148}$ = 395.8, $P$ < 0.0001) (Table 6).

### 3.4. Residual detection of propylene glycol from whole hams in the trial

There was no interaction ($P$ = 0.6130) between treatment and sampling position of hams. No difference existed ($P$ = 0.5350) in propylene glycol concentration between control and treatment hams ($F_{1, 38}$ = 0.39, $P$ = 0.5350) (Table 7). The concentration of propylene glycol in the treatment hams was 0.053% (53 mg/g), while the concentration for the control hams was 0.072% (Table 7). No differences existed among sampling position of hams ($F_{2, 38}$ = 0.18, $P$ = 0.8332), with the surface at 0.079%, middle point at 0.059%, and center at 0.055% (Table 7).

## 4. Discussion

### 4.1. Mold growth on whole hams from commercial facility

Each week, 10 hams were randomly selected in the sampling house which had approximately 100 control and 100 treatment hams, respectively in each lot. Hams in the later weeks such as week 7 or 11 did not have any mold, as they were not the same 10 sampling hams each week. Mold growth in aging facilities with

**Table 5**
Mite growth estimate on trial hams placed in an infested room with mites from previous whole ham experiments (more than 10,000 mites were released in the room in 2016, at 20–25 °C, 60–80% RH after aging approximately 9 mo at the commercial aging house (n = 3). Mean ± SE with the same letter within each column are not different (Tukey HSD at P = 0.05).

| ID | Estimate of mite growth | | | |
|---|---|---|---|---|
| | Lot A | Lot B | Lot C | Overall |
| Control | 1011 ± 504a | 1407 ± 512a | 989 ± 505a | 1136 ± 280a |
| Treatment | 187 ± 110b | 144 ± 61b | 259 ± 139b | 197 ± 63b |

Control = hams without being treated; Treatment = hams treated with PGA + CG + 40% PG coating.

**Table 4**
Mold growth estimate within each lot and each week on trial hams placed in an infested room with mites from previous whole ham experiments (more than 10,000 mites were released in the room in 2016, at 20–25 °C and 60–80% RH after aging approximately 9 mo at the commercial aging house (n = 3).

| Week | Treatment | Surface mold (%) | | | |
|---|---|---|---|---|---|
| | | Lot A | Lot B | Lot C | Overall |
| 1 | Control | 5.0 ± 3.1c | 29.2 ± 6.2bc | 1.3 ± 0.9d | 11.8 ± 3.7bc |
| 1 | Treatment | 0 ± 0c | 0 ± 0f | 0 ± 0d | 0 ± 0c |
| 5 | Control | 23.0 ± 6.1b | 44.2 ± 5.5a | 5.8 ± 1.4d | 24.3 ± 4.6a |
| 5 | Treatment | 2.9 ± 1.2de | 7.1 ± 3.2df | 2.5 ± 2.0d | 4.2 ± 1.3bc |
| 9 | Control | 33.3 ± 8.8 ab | 25.0 ± 3.8bcd | 29.2 ± 18.8bc | 29.2 ± 4.4 a |
| 9 | Treatment | 0.8 ± 0.8 c | 7.5 ± 6.3ef | 8.3 ± 6.0d | 5.6 ± 2.8bc |
| 10 | Control | 37.5 ± 7.3a | 20.0 ± 7.6cde | 33.3 ± 14.2 ab | 30.3 ± 5.8a |
| 10 | Treatment | 0.8 ± 0.8c | 7.5 ± 6.3ef | 13.3 ± 10.9cd | 7.2 ± 4.1bc |
| 12 | Control | 32.5 ± 11.5 ab | 38.3 ± 8.3 ab | 12.5 ± 3.8cd | 27.8 ± 5.8a |
| 12 | Treatment | 1.7 ± 1.7c | 8.3 ± 3.3def | 0.8 ± 0.8d | 3.6 ± 1.6bc |
| 13 | Control | 29.2 ± 4.4 ab | 40.8 ± 3.6 ab | 14.2 ± 7.1cd | 28.1 ± 4.7a |
| 13 | Treatment | 1.7 ± 1.7c | 8.3d ± 3.3ef | 0.8 ± 0.8d | 3.6 ± 4.9bc |
| 25 | Control | 33.3 ± 7.3 ab | 18.3 ± 4.4cde | 49.2 ± 9.8a | 33.6 ± 5.8a |
| 25 | Treatment | 0 ± 0c | 10.0 ± 5.8def | 5.0 ± 2.9d | 5.0 ± 2.4bc |

Mean ± SE with the same letter within each column are not different (Tukey HSD at P = 0.05).
Control = hams without being treated; Treatment = hams treated with PGA + CG + 40% PG coating.

**Table 6**
Mite growth estimate within each lot and each week on trial hams placed in an infested room with mites from previous whole ham experiments (more than 10,000 mites were released in the room in 2016, at 20–25 °C, 60–80% RH after aging approximately 9 mo at the commercial aging house (n = 3).

| Week | Treatment | Estimate of mite growth | | | |
|---|---|---|---|---|---|
| | | Lot A | Lot B | Lot C | Overall |
| 1 | Control | 0 ± 0d | 0 ± 0e | 0 ± 0d | 0 ± 0d |
| 1 | Treatment | 0 ± 0d | 0 ± 0e | 0 ± 0d | 0 ± 0d |
| 5 | Control | 0 ± 0d | 0 ± 0e | 0 ± 0d | 0 ± 0d |
| 5 | Treatment | 0 ± 0d | 0 ± 0e | 0 ± 0d | 0 ± 0d |
| 9 | Control | 0 ± 0d | 0 ± 0e | 0 ± 0d | 0 ± 0d |
| 9 | Treatment | 0 ± 0d | 0 ± 0e | 0 ± 0d | 0 ± 0d |
| 10 | Control | 0 ± 0d | 0 ± 0e | 0 ± 0d | 0 ± 0d |
| 10 | Treatment | 0 ± 0d | 0 ± 0e | 0 ± 0d | 0 ± 0d |
| 12 | Control | 567 ± 88 c | 2333 ± 333b | 450 ± 29c | 1117 ± 321c |
| 12 | Treatment | 42 ± 22d | 117 ± 17d | 183 ± 60cd | 114 ± 28d |
| 13 | Control | 533 ± 67c | 2333 ± 333b | 450 ± 29c | 1106 ± 323c |
| 13 | Treatment | 42 ± 22d | 117 ± 17d | 183 ± 60cd | 114 ± 28d |
| 25 | Control | 8000 ± 0a | 8000 ± 0a | 8000 ± 0a | 8000 ± 0a |
| 25 | Treatment | 1600 ± 529b | 1067 ± 272c | 1967 ± 769b | 1544 ± 310b |

Mean ± SE with the same letter within each column are not different (Tukey HSD at P = 0.05).
Control = hams without being treated; Treatment = hams treated with PGA + CG + 40% PG coating.

**Table 7**
The residual percentage from research trial whole ham samples of control and treatment nets and the position of sampling.

| Effect | Factor | Propylene Glycol Residual (%) |
|---|---|---|
| | Control | 0.072 ± 0.023 |
| Treatment | Treatment | 0.053 ± 0.017 |
| Position of sampling | Surface | 0.075 ± 0.032 |
| | Middle point | 0.059 ± 0.025 |
| | Center (near the bone) | 0.055 ± 0.018 |

Mean ± SE without any letter within each column are not different (Tukey HSD at P = 0.05).
Control = hams without being treated; Treatment = hams treated with PGA + CG + 40% PG coating.

whole hams is usually very sporadic, it was reasonable to see on week 7 and 11 that the 10 of the randomly chosen hams did not have any mold. Research in Italy indicated that mold growth was prevalent in maturing rooms, where hams were held at 14.9 °C and 62.4% RH for 2–7 months (Battilani et al., 2007). Additionally, research in Norway tested dry-cured meats aged between 4 and 22 months and isolated 264 molds from 161 samples (Asefa et al., 2009). This trial results demonstrate that the treated nets (with 1% PGA+1% CG+40% PG coating) inhibited mold growth on dry-cured hams in aging facilities. Results from the trial also indicated that not all lots of dry-cured hams became infested with mold. However, if the lot is susceptible or infested to mold, the coated nets were able to control the mold growth. Similar to these results Hendrix et al. (2018) tested ham slices wrapped in saturated netting under laboratory conditions and determined that nets saturated in solutions containing 40% PG reduced mold growth compared to untreated control solutions. Research by Portillo et al. (2018) on the use of fermentation by-products in food-grade coatings for dry-cured hams showed that solutions containing 10% PG effectively inhibited mold growth when used in direct contact applications. Krishnan et al. (2019) saturated nets with solutions containing 40% PG and wrapped around cubes of cave-aged cheddar cheese, which inhibited mold growth on the cubes. Those tests also showed that at 20 °C and both 75% and 85% RH, mold growth was excessive.

Canfield and Wrenn (2010) reported a positive association between mold growth and mite viability as mold acts as a shelter, free water, and a food source. Hendrix et al. (2018) also reported that the visual observation of mobile mites and the localization of their eggs near the fungal mycelium on ham slices in untreated nets. Yeasts (*Candida zeylanoides* and *Candida famata*, for example) have been shown to contribute to the sensory characteristics of dry-cured hams via lipolysis (Simoncini et al., 2007), and molds may be tolerated under certain conditions if the molds have an antioxidant effect to prevent surface degradation (becoming sticky or slimy), or contribute to lipolysis or proteolysis (Spotti et al., 2008). For example, *Penicillium chrysogenum* was shown to make significant contributions to proteolysis in the ripening of dry-cured meat products (Rodríguez et al., 1998). However, uncontrolled fungal growth can be detrimental, especially when leading to the production of allergenic compounds or mycotoxins (Spotti et al., 2008). *Penicillium* strains, such as *P. commune* and *P. polonicum* were isolated from ham production facilities in Spain and shown to produce cyclopiazonic acid (CPA), while *P. commune* and *P. verrucosum* can produce ochratoxin A (OTA), which is toxigenic (Alapont et al., 2014). Battilani et al. (2007) also identified *P. nordicum*, another OTA producer, as present in Italian ham facilities and these toxins can contribute to contamination of dry-cured hams.

### 4.2. Mold growth on whole hams from an infested simulated aging room

There were 16.9%–32.1% mold on the control hams surfaces, while the treatment hams had significantly lower surface mold ranging from 1.2 to 6.2%. In comparison, a study of two Spanish ham plants showed that mold growth was observed on 20–56% of post-salted hams (3 months), 20–40% of hams during ripening (3–7 months), and 40–60% of hams during aging (10–14 months) (Alapont et al., 2014). Rodríguez et al. (2012) collected 20 dry-cured Iberian hams at the beginning of the drying stage (6 months of ripening) for analysis and reported that ten of the twenty were contaminated with ochratoxin A (OTA), which indicates the presence of mold. Additionally, in a test of 65 commercially produced Spanish hams, Rojas et al. (1991) reported that 50% of hams sampled tested positive for *Aspergillus* and *Penicillium*. Asefa et al. (2010) tested deboned meat stored and aged in elastic netting and found that molds occurred from 0 to 100% throughout the production process post-smoking. The results in this research also concur with research by Zhang et al. (2018), which tested ham cubes wrapped in solutions containing propylene glycol and demonstrated that solutions containing propylene glycol were effective over long periods (up to 8 weeks) at controlling mold growth. Results of this research further demonstrated that hams with coating treated nets remained effective inhibiting mold growth on whole hams for 6 more months after 8 mo of aging in the simulated aging room.

### 4.3. Mite growth on whole hams from an infested simulated aging room

Whole ham infestation tests face multiple challenges. The net barrier and the muscle crevices and cracks of the ham, and the size of the sample, and the unknown numbers of mites that are still present in the environment of the simulated aging room, are all factors that influence the evaluation. Hendrix et al. (2018) reported that the CG + PGA+ 40% PG net treatments inhibited mite reproduction on dry-cured ham cubes (2.5 cm × 2.5 cm × 2.5 cm) and slices (2.5 cm × 9.0 cm × 15.5 cm) at all conditions (24, 28, 32 °C and 55, 65, 75 and 85% RH) and the 85% RH treatments showing the greatest level of mite inhibition. Mite residency on whole hams was tested with 900 mites, after 6 h of inoculation, 20% and 40% PG in Carrageenan and PGA had the lowest numbers

on those whole hams (Abbar et al., 2016). This effectiveness was also shown after 12 weeks on 40% PG of Carrageenan and PGA treatments, while the other treatments of 20 and 40% PG with xanthan gum did not reduce mite growth on whole hams 6 h after inoculation and 12 weeks later (Abbar et al., 2016). This agrees with the current research findings. The main differences between these studies are 1) that the current research was conducted in the aging room that was previously infested with approximately 16, 000 mites in 2016 and hams were not inoculated with mites in 2019 as the existing mite density was extremely high compared to a typical commercial infestation; 2) the current research incorporated the coating treatment of 40% PG with carrageenan and PGA treated polyester nets. The current research indicates that coated nets can be used to age and shape hams as well as control mold and mite growth. Campbell et al. (2017) stated that hams with coating-treated nets with higher absorbance reduced the rate of mite growth on whole hams, which was also confirmed in the current research. Therefore, these coating-treated nets will likely be effective at controlling mite infestations in commercial dry-cured ham plants.

### *4.4. Residual detection of propylene glycol from whole hams in the trial*

As reported above, the PG concentration in control and treatment ham did not differ, at an average of 0.0625% (62.5 mg/kg). This indicates that PG was not absorbed from the nets into the ham. No research has been conducted to quantify PG in dry-cured hams or meat. Wurita et al. (2013, 2014) discovered an appreciable concentration of PG in whole blood (8–689 ng/mL) and urine (59–5450 ng/mL) samples from healthy human subjects. The authors also reported that the consumption of 33.7 mg of PG in an energy drink increased the blood PG level by 158% in 0.5 h. However, there was only a 74% increase after 1 h. These findings indicate that PG is likely to come from the diets and it is metabolized quickly. A pig at market weight of 128 kg produces 53 kg of lean meat (National Pork Board, 2020); using 0.053% PG in treatment ham, with an average of 70 mL of blood per kg of bodyweight in pig (Hansard et al., 1951) and assuming that most PG comes to muscle from the bloodstream, the PG concentration in swine blood would be 3135 μg/mL. This is almost six times greater than what was reported in human blood because PG has been used as animal feed additives. Semi-moist pet foods often include humectants like propylene glycol to control water activity, reducing available water for fungal growth (Aldrich, 2006). PG and glycerol are also added to ruminant diets as energy additives (Ferraro et al., 2009). Moreover, with a significant amount of PG being excreted through urine and large fluctuation of PG concentration immediately after consumption of foods or drinks and with the similar PG concentration in both control and treatment hams, it is reasonable to conclude that most PG quantified in dry-cured hams were from the swine diet, not from treated nets. Analysis of fresh ham conducted to determine background PG yielded similar PG concentration (data not shown) in both control and treatment hams.

The concentration of 0.053% (53 mg/g) in the treatment hams is almost four times less than the maximum allowable concentration of this GRAS compound in food products (2%, FDA 21 CFR 184.1666, 2019). Although PG can be a potential health hazard when consumed over the recommended amount (25 mg/kg/day; Fowles et al., 2013; Mandl, 2018), the current concentration 0.053% (0.053 mg/kg) in treated hams makes it unlikely because it would require unrealistic daily consumption of more than 47 kg of dry-cured ham. Most dry-cured ham consumers in the United States do not eat more than a few slices, no more than 0.125 kg per meal on special occasions. A 2016 Survey by the National Pork Board of consumers indicated that ham is purchased an average of less than once per month, with most purchases occurring around Christmas and Easter (National Pork Board, 2016; National Pork, 2016). A serving size of 85 g–113 g per person was recommended due to the saltiness of country ham (Perry, 2011). According to the United States Department of Agriculture, Americans consume an average of 23.1 kg of pork products per year, with smoked ham being the most consumed processed pork product at 6.5 kg per year (Davis and Lin, 2005). The concentration in both the control and treatment hams (0.072 mg/kg and 0.053 mg/kg, respectively), which is much less than 2.0%, the maximum allowable concentration of this GRAS compound based on CFR 21,184.1666. These data, in addition to the argument above, indicate that the treated nets did not contribute to PG occurrence in the hams, that PG in dry-cured hams was minimal and potentially came from animal feed, and that the hams were safe for human consumption. Therefore, the net coating and the treated hams met legal requirements for commerce.

In summary, the research trial results demonstrated that the coating treated nets inhibited mold growth on whole hams in the commercial aging facility and analytical results showed that those hams are safe to consume, based on low propylene glycol concentration. The simulated aging house experiment with mites infested in the environment further validated that hams with coating treated nets were able to inhibit mites and mold from growing on the hams in comparison to the control hams over 6 months after approximately 8 mo of aging in the commercial facility. This indicates the long-term effectiveness of the nets at controlling mold and mite growth. Both types of research proved that these coating treated nets were effective at controlling mold and mite growth in ham aging facilities. Gas chromatography results showed that PG levels are minimal and dry-cured hams wrapped in these nets are safe for human consumption. Therefore, these nets may be considered a potential alternative to methyl bromide to control ham mite infestations for the dry-cured ham industry.

## CRediT authorship contribution statement

**Yan Campbell:** Data curation, Investigation, Formal analysis, Conceptualization, Funding acquisition, Project administration, Supervision, Writing - original draft. **Wenjie Shao:** Data curation, Investigation, Writing - review & editing. **Thu Dinh:** Formal analysis, Writing - review & editing. **Kezia To:** Data curation, Writing - review & editing. **William Rogers:** Writing - review & editing. **Xue Zhang:** Data curation, Writing - review & editing. **Thomas Phillips:** Conceptualization, Formal analysis, Funding acquisition, Writing - review & editing. **Wes Schilling:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Supervision, Writing - review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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