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The application of food grade short chain fatty acids to prevent infestation of *Tyrophagus putrescentiae* on dry cured ham and the effects on sensory properties

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ABSTRACT

Tyrophagus putrescentiae (ham mite) is difficult for commercial dry cured ham producers to control. Methyl bromide is an effective fumigant but is now banned as an ozone depleting substance, meaning alternative methods to control mite infestation must be found. This research was conducted to test the efficacy of $C_8C_9C_{10}$ fatty acids combined with and without food grade coatings to control mite infestations on dry cured hams. Ham cubes were coated directly or wrapped in nets saturated with $C_8C_9C_{10}$ with combinations of either soybean oil, xanthan gum (XG) or carrageenan (CG) + propylene glycol alginate (PGA). Cubes were then inoculated with 20 large mixed sex ham mites and stored for 14 days at 22 ± 2 °C and 70 ± 5% relative humidity. The soybean oil alone or in combination with 10% $C_8C_9C_{10}$ in direct coating, and 1% and 10% $C_8C_9C_{10}$ in coated nets controlled mite population growth. In addition, the use of 10% $C_8C_9C_{10}$ + XG and 10% $C_8C_9C_{10}$ + CG + PGA in direct coatings or in saturated nets, and 1% $C_8C_9C_{10}$ + XG in saturated nets also inhibited mite population growth. Unexpectedly, the soybean oil solvent by itself effectively controlled mite population growth as well. Sensory evaluation was performed using a difference from control test (n = 8) and indicated that only 10% $C_8C_9C_{10}$ mixed with soybean oil and 100% soybean oil did not impart sensory differences to ham when used as a coating. However, for ham slices treated in saturated nets and gum with $C_8C_9C_{10}$ mixtures in either coating or saturated nets did impart sensory differences. Results indicated that $C_8C_9C_{10}$ and soybean oil could be used in coating formulations to control ham mites but long-term testing, sensory evaluation, and scaled up testing is needed prior to industrial implementation.

1. Introduction

Dry-cured or American Country hams are aged and may be smoked but must lose at least 18% of their original weight (USDA FSIS 9 CFR 319.106, 2019). *Tyrophagus putrescentiae*(Schrank) (Sarcoptiformes: Acaridae), commonly referred to as the ham mite, mold mite or cheese mite, is one of the pests that may infest dry stored food products (Gulati and Mathur, 1995). Both *T. putrescentiae* and their feces are allergenic to people (Arlian et al., 1984). *T. putrescentiae* is also a vector of mycotoxin-producing fungi (Hubert et al., 2003). Therefore, mite contamination can pose a health risk in food products for consumers and the environment of plant workers (Hubert et al., 2003,2004). According to United States Department of Agriculture regulations, dry cured pork must not harbor mites, since this is considered adulteration (USDA FSIS 9 CFR 301.2). Controlling ham mites becomes particularly challenging as the aging process continues for the hams. **Integrated pest management (IPM) requires that ham aging rooms be cleaned and sanitized before hams are placed in storage. However, Rentfrow et al. (2008) reported that 22 out of 35 ham plants in the USA reported mite infestations, especially when hams were aged for more than five months. These plants varied in structural quality and cleanliness, with new plants with excellent sanitation also experiencing mite infestations.** Bromomethane, more commonly known as methyl bromide (CH_3Br), has been used as an effective fumigant for stored products since the 1930's, due to its rapid

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action and broad spectrum of activity (Fields and White, 2002). However, as environmental awareness has increased, there has also been increased scrutiny of substances that may damage the atmosphere. In the 1990's, the Montreal Protocol identified several ozone-depleting substances (ODS), including halons such as chlorofluorocarbons, carbon tetrachloride and methyl bromide (UNEP, 2019). The Montreal Protocol required that participating nations phase out the use of methyl bromide through 2015, which required a zero percent usage. The U.S. dry cured ham industry was granted a critical use exemption until 2017 (UNEP, 2019). At that time, it was determined that there were sufficient stockpiles of methyl bromide available in commerce to meet the needs of the U.S. dry cured pork industry without having to manufacture more. However, there are still concerns that cost-effective and environmentally safe alternatives to methyl bromide for ham mites are not yet available.

Research continues to investigate effective alternatives to methyl bromide fumigation as potential components of IPM. Controlled or atmospheric controls, such as reduced-oxygen atmosphere (ROA) (Sánchez-Molinero et al., 2010) and other gas mixtures such as high carbon dioxide (Hasan et al., 2016), essential oils from plants (De Assis et al., 2011), temperature and relative humidity (RH) control (Hendrix et al., 2018), and fumigant replacements like phosphine (Sekhon et al., 2010; Hasan et al., 2020) and sulfuryl fluoride (Abbar et al., 2018) have been tested for their efficacy at controlling mites, with mixed results. For example, a ROA required a RH of 50–60% to control mites, which is challenging to maintain. Phosphine was effective against mites but was corrosive to copper and electronics (Zhao et al., 2015), while sulfuryl fluoride was not effective at controlling mite eggs at greater than 3 times the label rate (Abbar et al., 2018). Food safe compounds such as xanthan gum (XG), propylene glycol (PG), carrageenan (CG), propylene glycol alginate (PGA) (Zhao et al., 2016), and lard have been tested as direct coating solutions (Abbar et al., 2016). These coatings with 10–50% PG for CG + PGA and 20–50% PG for XG were effective at controlling mite growth on dry cured ham cubes in laboratory conditions (Zhao et al., 2016). Coating whole hams with these solutions caused minimal sensory impact on the flavor, moistness, and texture of the dry cured ham at several aging facilities (Campbell et al., 2017). Unfortunately, the cost of additional processing and labor makes this less appealing. Therefore, the focus has shifted to include the use of polyester, cotton, and polyester/cotton blended nets that are currently used to hang hams during the aging process. Promising results have come from saturating these nets with solutions of XG or CG + PGA with 20–50% PG. These mixtures had no effect on the sensory characteristics of flavor, texture, and moistness of treated whole hams (Campbell et al., 2018).

Due to limited success in developing alternatives to methyl bromide for the control of *T. putrescentiae*, it is important to continue researching alternatives. Garlic juice is effective at controlling mites in laboratory studies, but is time-sensitive (Preisser et al., 2018) and imparts flavors that are uncharacteristic of dry-cured ham. Organic acids, including fatty acids, have been used as antimicrobial agents (Ricke, 2003). Short chain fatty acids are naturally occurring in the human body and are present in many food products. Specifically, $C_8C_9C_{10}$ (a mix of C8:0, C9:0 and C10:0 fatty acids) is generally recognized as safe (GRAS), environmentally friendly, and has been approved by the EPA as a fly repellant (Reifenrath, 2015). Dunford et al. (2016) tested a variety of $C_8C_9C_{10}$ concentrations mixed with silicone and acetone and determined that higher concentrations (over 75 μg/250 ml bottle) controlled test mosquitoes. Trials by Manu et al. (2018) showed that a proprietary blend of $C_8C_9C_{10}$ fatty acids was effective at controlling mite population growth on ham cubes. The objectives of this study were to (1) evaluate the effectiveness of the $C_8C_9C_{10}$ short chain fatty acid mixture as the active ingredient in a food grade coating to control *T. putrescentiae*, through direct contact and application to netting materials that are used in ham containment during storage and aging, and (2) evaluate the impact of $C_8C_9C_{10}$ short chain fatty acid mixture as the active ingredient in a food grade coating in both direct contact and saturated netting applications on the sensory properties of dry-cured ham.

2. Materials and methods

2.1. Food grade coatings materials

Carrageenan (Ticagel® 795 Powder, TIC Gums, White Marsh, MD), propylene glycol alginate (TICA-algin® PGA LV Powder, TIC Gums, White Marsh, MD), xanthan gum (Pre-Hydrated® Ticaxan® Rapid-3 Powder, TIC Gums, White Marsh, MD), propylene glycol (Hawkins, Roseville, MN), and a fatty acid blend $C_8C_9C_{10}$ (Emery Oleochemicals, Cincinnati OH) were used in the formulation of coatings for both direct contact and net saturation experiments. Ham stockinettes (152 loops per cm^3, 100% polyester) (Dickson Industries, Des Moines, IA) were cut into 14 cm squares and infused with mixed solutions.

2.2. Food grade coatings solution preparation

Coatings made with 1% xanthan gum (XG) were mixed with room temperature (25 °C) tap water before the addition of 10% propylene glycol (PG) or either 1% or 10% $C_8C_9C_{10}$ (a mix of C8:0, C9:0 and C10:0 fatty acids) fatty acids ($C_8C_9C_{10}$). Coatings made with 1% carrageenan (CG) and 1% propylene glycol alginate (PGA) and 10% PG or either 1% or 10% $C_8C_9C_{10}$ were solubilized in boiling water before cooling to approximately 65 °C. A 1% and 10% $C_8C_9C_{10}$ solution was also made with tap water. Soybean oil (Great Value, Wal-Mart Stores Inc. Bentonville, AR) was used as a solvent for 1% and 10% $C_8C_9C_{10}$ mixtures with no requirement for heating or cooling. Net solution pickups ranged from approximately 180 g/m^2 for water control samples to approximately 520 g/m^2 for XG infused samples. Samples using $C_8C_9C_{10}$ with soybean oil averaged approximately 330 g/m^2 pickup while soybean oil alone averaged approximately 370 g/m^2 pickup.

2.3. Ham slices and cube preparation

Commercial dry-cured hams weighing between 6 and 8 kg were sliced transversally into 2.5 cm thick slices using a band saw (B16–P, Butcher-Boy- Lasar MFG Company, Los Angeles, CA) and individually stored in vacuum packaging (40 cm × 60 cm, 3 mil. standard barrier, nylon/PE Clarity Vacuum Pouches, Koch Equipment LLC, Kansas City, MO; Model 2100 Vacuum Packager, Koch Equipment LLC, Kansas City, MO). The slices were then labeled and stored at 4 °C until use. The packages of ham slices were opened, and the ham was cut into 2.5 cm × 2.5 cm × 2.5 cm cubes in preparation for the mite infestation studies. Cubes were stored at 4 °C in vacuum bags (40 cm × 60 cm, 3 mil. standard barrier, nylon/PE Clarity Vacuum Pouches, Koch Equipment LLC, Kansas City, MO) until use. Ham cubes were randomly selected, tied with cotton strings and dipped into the following solutions: 1) water, 2) water +1% or 10% $C_8C_9C_{10}$, 3) 1% XG, 4) 1% XG + 10% PG, 5) 1% XG + 1% or 10% $C_8C_9C_{10}$, 6) 1% CG + 1% PGA, 7) 1% CG + 1% PGA +10% PG, 8) 1% CG + 1% PGA +1% or 10% $C_8C_9C_{10}$, 9) soybean oil, or 10) soybean oil +1% or 10% $C_8C_9C_{10}$ for 15 s prior to drying for 10 min. Cubes were also wrapped by nets saturated in the same respective solutions**. Each cube was individually wrapped with the corresponding saturated net, placed in**a 118 ml glass jar (**5.72 cm height, 6.35 cm diameter; Ball Corp., Broomfield, CO)**with black

construction paper inside the jar. **After inoculating the cube with 20 mites, the jar was sealed with the screw top portion of the lid, which secured a filter paper (**(Fisher Brand P4, Pittsburg, PA) at the top **in place of the metal lid so that mites were contained in the jar.**

2.4. Mite infestation assay

Tyrophagus putrescentiae cultures were reared and stored according to procedures provided by Abbar et al. (2016). Twenty mixed-sex adult mites were inoculated onto each ham cube from a laboratory colony. Cubes were stored in glass jars as detailed in section 2.3. Jars were stored in a basin containing a 0.5–1.0 cm high mixture of dish soap (Ajax Ultra, Colgate-Palmolive Company, New York, NY) and water with petroleum jelly (Vaseline, Unilever, Trumbull, CT) on the rim **as a means to prevent mites from escaping**. The basins were themselves stored in a locked storage cabinet at 22 ± 2 °C and 70 ± 5% relative humidity (RH) **to mimic the conditions of dry cured ham aging rooms**. Ham cubes and mites were incubated under these conditions for 14 days before removal for counting of mite progeny. After removal, each cube, black paper, jar, and netting (if applicable) was separated into individual petri dishes for evaluation. These items were examined using a 1.0–2.5 × optical microscope (Model 568, American Optical Company, Buffalo, NY) and mobile mites were counted.

2.5. Sensory analysis

2.5.1. Ham preparation

Coatings were made as described in section 2.2. Ham slices were prepared as detailed in section 2.3 and stored at 4 °C. Cotton thread was threaded through the bone section of a ham slice and used to suspend the slice during coating and drying. A 5 cm brush (Plaid Enterprises, Inc. Norcross, GA) was used to apply the respective solutions onto all visible surfaces of each slice. Stockinettes were cut to size and sewn back together to allow for better contact with each slice. Each net was tied at one end using cotton string before submerging into the respective solutions for saturation. After saturation, a ham slice was inserted into the stockinette and tied at the top using cotton string, which was also used to suspend the slice for drying. All hams slices were suspended to dry for 30 min before storage in gallon zip-top bags (Great Value, Wal-Mart Stores, Inc. Bentonville, AR) at 4 °C for 14 days.

2.5.2. Sensory evaluation

Ham slices were removed from refrigeration and equilibrated to room temperature (20–22 °C) for 30 min. All hams were removed from the zip-top bags and (if appropriate) stockinettes prior to rinsing with room temperature tap water (20 °C). Each slice was then fully wrapped in heavy duty aluminum foil (Reynolds Wrap, Reynolds Kitchens, Richmond, VA) and baked at 177 °C in an oven (Viking Corporation, Model #VGRC605-6G-SS, Greenwood MS) to an internal temperature of 71 °C to mimic dry-cured ham preparation in the United States (Zhao et al., 2016). Dry-cured ham does not need to be cooked and is predominantly consumed raw in Europe. However, it is most commonly cooked in the United States. Internal temperature was checked using a digital instant read thermometer (AcuRite, Lake Geneva, WI). After cooking, the slices were allowed to rest for 5–10 min. Ham slices were then cut into rectangular pieces that were 1.3 cm × 1.3 cm × 2.5 cm in size from the same muscle. Care was taken to preserve sample location and ensure that each panelist received samples from the same location on each ham slice in order to reduce variability between muscles. Samples were stored in 29.5 ml clear plastic containers (Dart Container Corporation, Mason, MI) that were labeled with random three-digit codes. Samples were randomly presented to trained panelists (n = 8) in a difference from control test. Panelists were provided with apple juice (Great Value, Wal-Mart Stores, Inc. Bentonville, AR), water, unsalted crackers (Great Value, Wal-Mart Stores, Inc. Bentonville, AR), and expectorant cups and were seated in separate booths during each panel. The difference from control test included a blind negative control to help determine the magnitude of any difference that existed. The test scale was as follows: 0 - no difference, 1 - slight difference, 2 - moderate difference, 3 - large difference, 4 - very large difference (Civille and Carr, 2015). Four separate tests were conducted that consisted of two replications with two subsamples per replication. Each test included either four or five samples of either direct contact coatings or saturated netting hams: a labeled control, a blind control, 1% XG with 10% $C_8C_9C_{10}$, 1% CG with 1% PGA and 10% $C_8C_9C_{10}$, and 10% $C_8C_9C_{10}$ with soybean oil.

2.6. Statistical analysis

A randomized complete block design with 3 replications, that were performed at 3 separate times, and five sub-samples each, for both direct contact and saturated polyester netting, was used to test the effects of incorporating the $C_8C_9C_{10}$ fatty acid into XG, PGA + CG, water, and soybean oil on mite infestations on dry cured ham. Controls of water, XG, XG + PG, CG + PGA, CG + PGA + PG, and soybean oil were included for comparison regarding mite growth and reproduction.

A randomized complete block design with two replications and two subsamples was used for the difference from control tests. Data collection was performed using Compusense software (Compusense Cloud, Guelph, CA). The one-way analysis of variance (ANOVA) method was used to determine if there were significant differences ($P < 0.05$) among treatment means (SAS version 9.4, SAS Institute, Cary, NC) according to established statistical methodology in Civille and Carr (2015). For treatments where differences ($P < 0.05$) occurred, Tukey's Honestly Significant Difference Test ($P < 0.05$) was used to separate treatment means.

3. Results

3.1. Mite infestation study

3.1.1. $C_8C_9C_{10}$ with water or soybean oil

For coated samples, 10% $C_8C_9C_{10}$ in water had fewer mites ($F_{5,63} = 9.71$, $P < 0.0001$) than the water control but did not differ ($P > 0.05$) from any other treatments (Table 1). Interestingly, the treatment with 100% soybean oil had fewer mites ($P < 0.0001$) than the 100% water and 1% $C_8C_9C_{10}$ in water treatments and did not differ ($P > 0.05$) from any other treatments (Table 1). Only the soybean oil control and the 10% $C_8C_9C_{10}$ mixed with soybean oil resulted in mite counts near or below the initial inoculation levels

Table 1
Tyrophagus putrescentiae counts (n = 15) on dry cured ham cubes (2.54 cm × 2.54 cm × 2.54 cm) that were directly coated or wrapped in saturated nets with $C_8C_9C_{10}$ mixed with water or soybean oil and inoculated with 20 mixed sex mites and incubated for 14 days at 22 ± 2 °C and 70 ± 5% RH.

Treatment	Mean No. Mites- Coating	Mean No. Mites- Nets
100% Water	$285.9^{a} \pm 35.5$	$78.7^{a} \pm 16.7$
100% Soybean oil	$21.0^{c} \pm 9.3$	$0.0^{b} \pm 0.0$
1% $C_8C_9C_{10}$, 99% Water	$210.9^{ab} \pm 22.5$	$34.1^{ab} \pm 13.2$
10% $C_8C_9C_{10}$, 90% Water	$108.5^{bc} \pm 33.3$	$24.1^{ab} \pm 12.1$
1% $C_8C_9C_{10}$, 99% Oil	$76.7^{bc} \pm 33.2$	$0.2^{b} \pm 0.1$
10% $C_8C_9C_{10}$, 90% Oil	$13.5^{c} \pm 5.6$	$0.0^{b} \pm 0.0$

[abc] Means with the same superscript in a column indicate no difference ($P > 0.05$).

of 20 for direct contact application. When using saturated nets, the 100% soybean oil, the 1% $C_8C_9C_{10}$ in soybean oil and the 10% $C_8C_9C_{10}$ in soybean oil had fewer mites than the water control ($F_{5,63} = 4.46$, $P = 0.0015$), and no other differences ($P > 0.05$) existed (Table 1). The lack of statistical differences between treatments can be partially attributed to large variation in mite counts for each treatment.

3.1.2. $C_8C_9C_{10}$ with xanthan gum

For the coating experiment, ham cubes treated with 1% XG + 10% $C_8C_9C_{10}$ had 6 mites per cube on average, which was fewer ($F_{3,56} = 4.22$, $P = 0.0093$) than the 1% XG + 99% water and the 1% XG + 1% $C_8C_9C_{10}$ treatments (Table 2). When directly coated, there was no difference ($P > 0.05$) between cubes treated with XG, XG + 10% PG, and XG + 1% $C_8C_9C_{10}$ with all treatments having mites above the initial inoculum level of 20 mites. When using saturated polyester netting, there was no difference ($P > 0.05$) between cubes treated with XG + 10% PG and XG + 1% or 10% $C_8C_9C_{10}$. In addition, the XG + 1% or 10% $C_8C_9C_{10}$ had fewer mites ($F_{3,56} = 6.19$, $P = 0.0010$)than XG alone.

3.1.3. $C_8C_9C_{10}$ with carrageenan and propylene glycol alginate

In direct coating applications, the use of CG + PGA +10% $C_8C_9C_{10}$ had fewer mites ($F_{3,56} = 7.81$, $P = 0.0002$) than other treatments and the mite count was below the initial inoculation level of 20 mites after 14 days of incubation (Table 3). In saturated nets, CG + PGA +10% $C_8C_9C_{10}$ was the most effective net saturation solution for controlling mite population growth (less than 1 mite/cube on average) but was not statistically different ($P > 0.05$) from the CG + PGA + 10% PG treatment ($P > 0.05$) (Table 3). The gum (CG and PGA) alone and CG + PGA + 1% $C_8C_9C_{10}$ treatments had more mites ($F_{3,56} = 4.12$, $P = 0.0103$) than the CG + PGA + 10% $C_8C_9C_{10}$ treatment, but did not differ ($P > 0.05$) from CG + PGA + 10% PG. Results indicate that $C_8C_9C_{10}$ may be a potential alternative to PG as the active ingredient in CG + PGA based coatings as a deterrent to mite growth.

3.2. Sensory evaluation

In direct coating applications, the soybean oil +10% $C_8C_9C_{10}$ treatment was not different ($P > 0.05$) from the blind control with respect to texture and flavor ratings reported by the panel but was different ($F_{3,51} = 4.87$, $P = 0.0047$) with respect to moistness (Table 4). The XG + 10% $C_8C_9C_{10}$ and CG + PGA +10% $C_8C_9C_{10}$ treatments differed ($P < 0.05$) from the blind control regarding texture ($F_{3,51} = 4.87$, $P = 0.0047$), flavor ($F_{3,51} = 12.41$, $P < 0.0001$), and moistness ($F_{3,51} = 4.87$, $P = 0.0047$). The flavor of hams that were coated with gum and 10% $C_8C_9C_{10}$ was moderately or largely different from the control, which might be due to the addition of $C_8C_9C_{10}$ (Table 4), since $C_8C_9C_{10}$ is a volatile mix of fatty acids with distinct aromas. When placed in polyester nets, the soybean oil +10% $C_8C_9C_{10}$ and CG + PGA +10% $C_8C_9C_{10}$ treatments were different than the blind control with respect to texture ($F_{3,52} = 4.57$, $P = 0.0065$), flavor ($F_{3,52} = 25.4$, $P < 0.0001$), and moistness ($F_{3,52} = 6.56$, $P = 0.0008$) (Table 4). The XG + 10% $C_8C_9C_{10}$ treatment was different from the blind control with respect to flavor and moistness ($P < 0.05$) but was not different ($P > 0.05$) with respect to texture.

Since soybean oil had efficacy at controlling mite population growth when no $C_8C_9C_{10}$ was included in the coating (Table 1), additional difference from control tests were conducted to determine its impact on the sensory properties of the ham. The dry cured ham slice that was coated directly in 100% soybean oil or soybean oil +10% $C_8C_9C_{10}$ were not rated differently from the blind control with regards to texture ($F_{2,14} = 1.92$, $P = 0.1832$), flavor ($F_{2,14} = 2.43$, $P = 0.1238$), and moistness ($F_{2,14} = 0.81$, $P = 0.4663$)(Table 5). However, despite the lack of statistically significant differences, the difference values from the blind control were numerically higher for the 10% $C_8C_9C_{10}$ treatment when compared to 100% soybean oil alone. In contrast, 100% soybean oil in nets differed ($F_{2,14} = 5.19$, $P = 0.0206$) in texture, and soybean oil + 10% $C_8C_9C_{10}$ in nets differed ($P < 0.05$) in flavor ($F_{2,14} = 10.72$, $P = 0.0015$) in comparison to the blind control (Table 5).

4. Discussion

The 1% $C_8C_9C_{10}$ in water did not successfully control mite reproduction, with counts higher than the initial inoculation level. This is largely due to a combination of the hydrophobic nature of the $C_8C_9C_{10}$ and the low concentration of $C_8C_9C_{10}$ not dispersing well in solution, which contributed to variability in $C_8C_9C_{10}$ concentration throughout the coating. The use of 100% soybean oil solution was effective at controlling mites and no mobile mites were found after 14 days of incubation. The effectiveness of soybean oil was similar to that of lard, as reported by Zhao et al. (2016), who concluded that lard as a direct coating and 100% PG were effective at controlling mites on ham cubes for 14 and 21 d on 15.6 cm^3 ham cubes that had been inoculated with 20 mites. However, the effectiveness of soybean oil was different to that reported by Abbar et al. (2016), whose tests included soybean oil as a direct coating. That study was performed on 15.6 cm^3 ham cubes at 25 °C and 70% RH, with results for soybean oil nearing at 197 mites. The two specific differences in these researchers testing in comparison to the current study was that their temperature was slightly greater at 25 °C in comparison to 22 °C used here and their jar was relatively twice as large at 216 ml vs 119 ml. Inconsistent results were also found in studies with lard as a direct coating or in saturated nets to control mites. In comparison with Zhao et al. (2016), Zhang et al. (2018) reported that the use of lard on saturated netting was ineffective at controlling mites. In addition, Garcia (2004) reported that Iberian ham producers use manual cleaning of hams followed by submersion into hot lard to control mites. In the current study, solutions with soybean oil alone or in combination with 1% or 10% $C_8C_9C_{10}$ controlled infestation to practically zero mites when using saturated nets (Table 1). This implies that soybean oil

Table 2
Tyrophagus putrescentiae counts (n = 15) on dry cured ham cubes (2.54 cm × 2.54 cm × 2.54 cm) that were directly coated or wrapped in saturated nets with $C_8C_9C_{10}$ mixed with xanthan gum (XG) and inoculated with 20 mixed sex mites and incubated for 14 days at 22 ± 2 °C and 70 ± 5% RH.

Treatment	Mean No. Mites- Coating	Mean No. Mites- Nets
1% XG, 99% Water	61.1[a] ± 15.2	75.0[a] ± 21.8
1% XG, 10% PG, 89% Water	45.1[a] ± 10.8	29.8[ab] ± 8.7
1% XG, 1% $C_8C_9C_{10}$, 98% Water	54.0[a] ± 15.7	14.5[b] ± 4.6
1% XG, 10% $C_8C_9C_{10}$, 89% Water	6.0[b] ± 3.0	6.5[b] ± 5.8

[ab] Means with the same superscript in a column indicate no difference ($P > 0.05$).

Table 3
Tyrophagus putrescentiae counts (n = 15) on dry cured ham cubes (2.54 cm × 2.54 cm × 2.54 cm) that were directly coated or wrapped in saturated nets with $C_8C_9C_{10}$ mixed with carrageenan (CG) and propylene glycol alginate (PGA) and inoculated with 20 mixed sex mites and incubated for 14 days at 22 ± 2 °C and 70 ± 5% RH.

Treatment	Mean No. Mites- Coating	Mean No. Mites- Nets
1% CG, 1% PGA, 98% Water	68.5^{a}± 16.5	45.1^{a}± 10.1
1% CG, 1% PGA, 10% PG, 88% Water	96.2^{a}± 18.2	23.1^{ab} ± 6.3
1% CG, 1% PGA, 1% $C_8C_9C_{10}$, 97% Water	89.3^{a}± 12.1	44.3^{a}± 7.5
1% CG, 1% PGA, 10% $C_8C_9C_{10}$, 88% Water	10.3^{b} ± 5.5	0.7^{b} ± 0.4

[ab] Means with the same superscript in a column indicate no difference (P > 0.05).

Table 4
Sensory differences in texture, flavor, and moistness of 2.54 cm thick ham slices that were directly coated or wrapped in saturated nets with 10% $C_8C_9C_{10}$ solutions using a 5-point difference from control test (n = 8) after 14 days of storage at 2–4 °C.

Treatment	Direct Contact			Saturated polyester nets		
	Texture	Flavor	Moistness	Texture	Flavor	Moistness
100% Water (Blind control)	0.6^{b} ± 0.18	1.0^{c}± 0.18	0.5^{b} ± 0.13	0.3^{b} ± 0.10	0.4^{b} ± 0.18	0.1^{b} ± 0.09
1% XG, 10% $C_8C_9C_{10}$, 89% Water	1.6^{a}± 0.28	2.3^{ab} ± 0.26	1.5^{a}± 0.25	1.3^{ab} ± 0.24	2.6^{a}± 0.20	1.1^{a}± 0.26
1% CG, 1% PGA, 10% $C_8C_9C_{10}$, 88% Water	1.6^{a}± 0.25	3.0^{a}± 0.18	1.3^{a}± 0.23	1.4^{a}± 0.31	2.5^{a}± 0.22	1.3^{a}± 0.30
10% C8C9C10, 90% Oil	1.4^{ab} ± 0.24	1.6^{bc}± 0.21	1.3^{a}± 0.21	1.4^{a}± 0.21	1.9^{a}± 0.21	1.1^{a}± 0.17

[ac] Means with the same superscript in a column indicate no difference (P > 0.05).
Scale for difference from control test- 0 = no difference, 1 = slight difference, 2 = moderate difference, 3 = large difference, 4 = very large difference.

Table 5
Sensory differences in texture, flavor, and moistness of 2.54 cm thick ham slices that were directly coated or wrapped in saturated nets with soybean oil treatments stored at 2–4 °C for 14 days using a 5-point difference from control test (n = 8).

Treatment	Direct Contact			Saturated polyester nets		
	Texture	Flavor	Moistness	Texture	Flavor	Moistness
100% Water (Blind Control)	0.5^{a}± 0.30	0.9^{a}± 0.33	0.8^{a}± 0.20	0.6^{b} ± 0.21	0.8^{b} ± 0.35	0.6^{a}± 0.21
100% Soybean oil	0.6^{a}± 0.37	0.9^{a}± 0.33	1.0^{a}± 0.38	2.1^{a}± 0.53	1.9^{ab} ± 0.49	1.8^{a}± 0.62
10% $C_8C_9C_{10}$, 90% oil	1.3^{a}± 0.19	1.9^{a}± 0.44	1.4^{a}± 0.41	1.5^{ab} ± 0.36	3.1^{a}± 0.33	1.1^{a}± 0.42

Scale for difference from control test- 0 = no difference, 1 = slight difference, 2 = moderate difference, 3 = large difference, 4 = very large difference.
[ab] Means with the same superscript in a column indicate no difference (P > 0.05).

(the solvent in the study) may be effective at controlling mites and further testing is warranted on its long-term effectiveness. Another important result of this study is that ham cubes wrapped in a saturated polyester net had lower numbers of mites when compared to cubes that were coated directly with corresponding solutions. This is due to the net itself acting as a physical barrier to mite movement, largely due to its small mesh size.

The use of polyester nets saturated with XG + 1% or 10% $C_8C_9C_{10}$ resulted in mite counts below inoculation levels. While not exactly matching prior studies, these results follow the trend that XG alone is ineffective at controlling mite growth, while the addition of PG along with XG slowed mite growth. Zhang et al. (2017) tested both mite orientation/oviposition and reproduction for ham cubes wrapped in nets saturated with XG + PG or CG + PGA + PG. These researchers reported that gum-only saturated nets were ineffective at controlling mite growth, but positive results were gained by adding medium to high concentrations (40–50%) of PG. These results are also similar to those of Campbell et al. (2017), who reported that 15% PG was needed to control mites when mixed with XG. Therefore, it was not expected for the 10% PG treatment to be effective in the current study. Results indicate that the XG + 10% $C_8C_9C_{10}$ treatment either in direct contact or in saturated nets may be an effective treatment for controlling mite growth (Table 2). However, from the previous experiment (Table 1), it appears that soybean oil may be a better carrier for $C_8C_9C_{10}$ than XG mixed with water because there were no mobile mites after 14 days of incubation for soybean oil or soybean oil + 10% $C_8C_9C_{10}$ treatments in saturated nets. The disadvantage of the soybean oil is that it may not remain effective on the surface of the ham for months of aging since it is liquid and not a solid coating. Therefore, it is necessary to evaluate the effectiveness of soybean oil and soybean oil + $C_8C_9C_{10}$ on mite growth over a longer storage period. The addition of a stabilizer as a means of delivery may help increase long-term effectiveness.

Research from the current study and research reported from Manu et al. (2018) showed that $C_8C_9C_{10}$ was effective at controlling *T. putrescentiae* under laboratory conditions. Reifenrath (2015) reported that $C_8C_9C_{10}$ is categorized as "generally recognized as safe" by the US. S. Food and Drug Administration and is registered with the U.S. Environmental Protection Agency as an insect repellant against ticks, flies, and mosquitoes. Even though the mechanism of $C_8C_9C_{10}$ with respect to repellency and insecticidal properties are unknown, its effectiveness against ticks, flies, and mosquitoes is possibly due to its volatility and minimum evaporation rate (Skinner and Johnson, 1980). Shorter chain fatty acids than $C_8C_9C_{10}$ are too volatile and therefore evaporate into the environment, and longer chain fatty acids are not volatile enough to repel pests. Mullens et al. (2009) showed that 15–30% $C_8C_9C_{10}$ was able to control horn flies on cattle, and Reifenrath (2015) reported that 15% $C_8C_9C_{10}$ in kaolin was as or more effective than DEET at controlling *Aedes aegypte*, the yellow fever mosquito. In addition, Dunford et al. (2016) reported that $C_8C_9C_{10}$ showed potential for controlling *Aedes aegypte* and *Aedes albopticus*, which are common mosquito vectors for dengue and Zika virus. These authors also stated that additional testing would need to be conducted on wild captured mosquito strains to ensure that the mosquitoes die after treatment. Results from the current study indicate that *T. putrescentiae* control is also a potential application for $C_8C_9C_{10}$.

The differences between the $C_8C_9C_{10}$ + soybean oil treatment that existed with polyester nets but not in the direct coating may be due to the volatile compounds associated with the $C_8C_9C_{10}$ treatment becoming trapped in the nets and oxidation of the fatty acids over time. This also may be due to nets absorbing a greater amount of coating than coated samples without nets. It may be possible that the $C_8C_9C_{10}$ fatty acids are combining with volatile compounds that are present in the ham to produce aromas that are trapped by the netting and perceived negatively by the taste panel. These results suggest that both the 10% $C_8C_9C_{10}$ in soybean oil and the 100% soybean oil alone are the only possible treatments that were evaluated that could potentially be used to control mites without causing sensory differences in the hams. Sensory testing from Campbell et al. (2017) differed from this study in that whole hams were treated in the industry with coatings of gum + PG, not slices as was done in the current study. Similar results and values were seen in the current study with respect to coating whole hams by dipping in Campbell et al. (2017), with mean values of attributes were slightly different from the controls. However, when coating was sprayed on the hams, there were no sensory differences between coated and uncoated samples. This demonstrates that coating application method can impact the sensory properties of the hams and that spraying is better than coating with respect to costs and impact on sensory properties. Campbell et al. (2018) also reported that treating whole hams with coatings or placing them in coated nets and aging them for 4 months did not impart sensory differences in the hams. This demonstrates that treating whole hams has less impact than treating ham slices with coating and warrants future sensory testing of whole hams that have been coated with $C_8C_9C_{10}$.

In conclusion, while the $C_8C_9C_{10}$ blend has been tested in both laboratory and field tests for insect repellency, this is one of the first studies exploring its effectiveness at controlling *Tyrophagus putrescentiae* growth. These laboratory tests have shown that 100% soybean oil alone and a solution including 10% $C_8C_9C_{10}$ in soybean oil, 1% XG or 1% CG + 1% PGA were effective at controlling ham mite population growth as either a direct contact solution or when used in saturated polyester nets. Dry-cured ham slices that were treated with soybean oil, either alone (100%) or mixed with 10% $C_8C_9C_{10}$, were not different from a blind control in direct contact applications with respect to sensory properties, but did exhibit variability from the blind control when used with saturated polyester nets. This is possibly due to the nets trapping volatile compounds rather than releasing them. Further study is recommended for $C_8C_9C_{10}$ and gum mixtures or soybean oil along with other oils based on both mite population control and sensory results, including long-term mite infestation testing for months, sensory evaluation on whole hams that are coated or sprayed with recommended $C_8C_9C_{10}$ mixtures, and scaled up testing prior to industrial implementation.

CRediT authorship contribution statement

William Rogers: Data curation, Investigation, Writing - original draft. **Yan L. Campbell:** Funding acquisition, Conceptualization, Writing - review & editing. **Xue Zhang:** Formal analysis, Project administration, Writing - review & editing. **Wenjie Shao:** Data curation, Validation, Writing - review & editing, Conceptualization, Formal analysis, Funding acquisition, Project administration, Supervision, Writing - review & editing. **Shecoya White:** Writing - review & editing. **Thomas W. Phillips:** Funding acquisition, Conceptualization, 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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