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Application of food grade coatings to prevent mite infestations in dry cured ham
processing facilities

By

Yan Li Campbell

A Dissertation
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Food Science
in the Department of Food Science, Nutrition and Health Promotion

Mississippi State, Mississippi

December 2017

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2017

Application of food grade coatings to prevent mite infestations in dry cured ham
processing facilities

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The ham mite, *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae) is the predominant pest of dry cured hams during aging in the processing facilities. Methyl bromide is currently the only known fumigant that is effective at controlling ham mite infestations in aging houses. However, methyl bromide is being phased out of all industries and will be depleted in the near future. The research objectives were to 1) evaluate dry cured hams that have been treated with previously developed food grade coatings for sensory differences, and 2) to develop and determine the efficacy of ham nets incorporated with food grade coatings on controlling mite infestations and sensory properties. Food grade coating combinations of 1) propylene glycol (PG), xanthan gum, and water or 2) PG, propylene glycol alginates, carrageenan and water were dipped and sprayed on whole hams in commercial facilities in the summers of 2014 and 2015 (composition patent pending). The lowest concentration of propylene glycol needed to control mites in laboratory studies was 15% with xanthan gum and 7.5% with propylene glycol alginate and carrageenan. Sensory difference from control tests with trained panelists indicated that there were slight to moderate differences detected in some of the

treated hams in comparison to untreated control hams ($P < 0.05$) when hams were dipped with coatings. However, there were no differences ($P > 0.05$) detected between the treated hams and the control hams when hams were only sprayed rather than dipped with these coatings. Polyester/cotton blend or cotton nets were infused with various food grade coatings and evaluated on the bench top by inoculating 20 adult mites onto one inch ham cubes for their efficacy at controlling mite infestations. Live adults and mobile immature stages were counted after 14 d of incubation (23 ± 2 °C and $70 \pm 5\%$ RH). Mite infestation tests demonstrated that coatings and coating-treated nets were effective at controlling mite growth. Therefore, food grade coatings can be applied to dry cured hams and also can be infused into nets as a potential means to control mite growth in ham processing facilities.

DEDICATION

I would like to dedicate this manuscript to my husband Jonathan Doss Campbell, my daughter Mei Lynn Campbell, my parents Xueqi Li and Juxiang Yao, and my families in both China and the United States. Without your sacrifices, support, encouragement and love, I could not have accomplished this. We did it together!

Love,

Yan

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CHAPTER I

INTRODUCTION

The settlers of Jamestown, Virginia started producing dry-cured hams in the early 1600s when they learned how to preserve raw meat with salt from the Native Americans (Stradley, 2004). In addition, some Europeans migrated to America and brought their cultural traditions of dry curing hams. American dry cured ham was adapted from Westphalian-style hams from Germany, Iberian hams from Spain, Prosciutto-style hams from Italy, and dried meats from the Native Americans (Rentfrow et al., 2012). American dry-cured ham must lose at least 18% of its original weight during curing and contain a minimum of 4% salt (USDA 9 CFR 319.106). The characteristic flavor and texture of hams comes from extensive lipolysis and proteolysis (Toldrá & Flores, 1998). Country hams are produced in the southeastern states of Georgia, Tennessee, Kentucky, North Carolina, and Virginia as well as the Midwestern State of Missouri.

Tyrophagus putrescentiae, also known as the ham mite, cheese mite, or mold mite, is the predominant target pest for dry-cured ham. Ham mites are frequently found in a wide variety of stored food products, particularly those with high fat and protein content, such as hams, dried eggs, bacon, flour, herring meal, cheese, different kinds of nuts (Hughes, 1976), and stored grains (Griffiths et al., 1976; Van Hage-Hamstem & Johansson, 1992). Ham mites feed on the surface of dry cured meat and follow the seams between muscles to the interior of the hams. They crawl on the ham extensively and may

be carried from one place to another by humans or other insects. A female can reproduce up to 800 eggs on the surface of the ham in a 2-1/2 week period during the summer (Townsend, 2008). Regulations do not allow dry-cured hams to have any mites on the surface of the hams (USDA 9 CFR 301). Therefore, any mite that is present on the hams will need to be controlled.

Methyl bromide has been used by dry cured ham processing plants to control and prevent ham mite infestations for greater than 50 years (Fields & White, 2002). Methyl bromide is a broad spectrum fumigant that is the only known available fumigant that is effective at controlling ham mite infestations in aging facilities. However, this fumigant contributes to the depletion of the stratospheric ozone layer (Marriott & Schilling, 2004) and will be phased out of all industries by 2015 according to the Montreal Protocol, an international agreement ratified by more than 180 countries. There are at least 35 dry cured ham plants in the United States that are located in Kentucky, Missouri, North Carolina, Virginia, Tennessee, and Georgia. Twenty-two of these plants used methyl bromide as a fumigant between one to five times a year prior to 2008 (Rentfrow et al., 2008). The number of times that fumigation occurred was due to the number of infestations. Country ham processors are permitted to have access to methyl bromide until the existing stocks are depleted (EPA, 2015a). Therefore, it is critical for the dry-cured ham industry to find viable, effective and economical alternatives to replace methyl bromide to control mite infestations.

Prior to 2006, research on methyl bromide alternatives for dry-cured ham was minimal. Fumigants such as phosphine and sulfuryl fluoride (Sekhon et al., 2010a; 2010b), physical control methods such as modified atmosphere (Sánchez-Molinero et al.,

2010), ozone (Hassan et al., 2016), and heating (Abbar et al., 2016a) have been evaluated. In addition, pesticides and bioactive compounds such as Storcide II® and limonene from pine essential oils (Abbar et al., 2017) have been investigated for their efficacy at controlling infestations. Zhao et al. (2016a) evaluated food grade coatings, including food oils and synthetic polyols for their efficacy at controlling mites. Results indicated that 100% lard and 50% or 100% propylene glycol prevented mite reproduction on treated ham pieces, while vegetable oils (corn, soybean and olive), potassium sorbate and glycerol had minimal effects on controlling ham mites.

Food-grade coatings have been used to prevent water loss and reduce rancidity in meat products (Baldwin, 2007). However, dry cured hams must lose at least 18% of its original weight during aging (USDA 9 CFR 319.106), and oxygen exposure is needed for proteolysis and lipolysis, which results in the development of the unique flavors of dry cured ham (Toldrá & Flores, 1998). Propylene glycol is relatively expensive (\$16/liter). Therefore, food grade coatings that contain xanthan gum, propylene glycol alginate, carrageenan and propylene glycol were developed to control mite growth on dry-cured ham cubes as well as to maintain water vapor and oxygen permeability (Zhao et al., 2016a). The polysaccharides were used in the coatings to prevent propylene glycol from evaporating and to stabilize and lock the active ingredient in the gel matrix so that it remains on the surface of the hams.

Zhao et al. (2016a) demonstrated that food grade coatings that contained PG were effective at inhibiting ham mite reproduction on $2.54 \times 2.54 \times 2.54$ cm ham cubes. Concentrations of 10 and 20% PG were needed to control mite infestations for the propylene glycol alginate + carrageenan based coating and the xanthan gum based

coating. However, there was no report on how the coatings affected the sensory properties when applied on whole hams. The first objective of this study was to determine the most cost effective concentrations of PG that can be included in these coatings and effectively control mites. The second objective of this research was to apply coatings to whole hams in commercial ham processing plants and determine their impact on sensory properties.

In the textile industry, treating bedding fabrics and encasement to reduce house dust mite allergens has been investigated extensively and the application has been utilized in the bedding industry to reduce allergic reactions. For example, anti-mite modified spun polypropylene fibers with ceramite and bedding inserts containing such fibers were evaluated for their efficacy at controlling mites and allergic reactions (Niekraszewicz et al., 2005). Nishioka et al. (1997) investigated the use of a bedding encasement with mite blocking fibers with atopic dermatitis in infants and concluded that the bedding encasement was effective at preventing atopic infants from being sensitized to house dust mites, including *T. putrescentiae*. Borkow and Gabbay (2004) and others incorporated copper into textiles and fabrics to produce anti-viral masks, gloves and filters to protect from HIV-1, flu and other viruses, and anti-bacterial fabrics to destroy antibiotic resistant bacteria (Gabbay et al., 2006), and anti-fungal socks to treat athlete's foot (Zatcoff et al., 2008).

Bioactive compounds can also be placed in food-grade coatings and infused into stockinettes that dry-cured ham processors in the United States currently use in order to control mite infestations. Stockinettes have been used for meat packaging for a long time (Claxton, 1919). Treating stockinettes with liquid smoke, oils, acid solutions is a common

practice in the meat industry to enhance peelability of the nets or the color or flavor of the meat product. Coating whole hams creates additional processing steps and labor as well as requires a greater amount of coating materials to cover the whole ham. Thus, the second objective of this study was to infuse food grade coatings developed by Zhao et al. (2016a) into different types of ham stockinettes (ham nets) to evaluate their efficacy at controlling mite growth as well as their impact on the sensory properties of the hams.

CHAPTER II

LITERATURE REVIEW

2.1 History of Dry-cured Ham

Marine salt with nitrate as an impurity was first used to preserve meat prior to 2000 B.C. The production of dry-cured pork in southern European Mediterranean countries can be traced back to 2000 BC (Toldrá, 2002). For centuries, the evolution of dry cured ham followed the traditional route over centuries by communicating the process from one generation to the next (Toldrá, 1992; Toldrá et al., 1997). Some of the most well-known hams that have been produced in the 20th and 21st centuries include the Iberian and Serrano hams from Spain, Bayonne and Corsican hams from France, Parma and San Danielle hams from Italy, Jin Hua and Yunnan hams from China, Katenschinken and Westphalia hams from Germany, Sauna hams from Finland, and Country-style hams from the United States (Toldrá, 2004).

American dry-cured hams, also known as country hams were adapted from the dry-cured hams from the south Europe Mediterranean countries (Rentfrow et al., 2012) when Europeans migrated to current day America with their dry curing ham traditions. The settlers of Jamestown started producing hams in the early 1600s by using knowledge from previous generations and the Native Americans, who used salt to preserve meat (Stradley, 2004). By law, American dry-cured ham must lose at least 18% of its original weight during production and contain a minimum of 4% salt (USDA 9 CFR 319.106).

The characteristic flavor and texture come from the extensive proteolysis and lipolysis that occurs during the aging process (Toldrá & Flores, 1998). The majority of country hams are produced in the southeastern states of Georgia, Tennessee, Kentucky, North Carolina, Virginia and the Midwestern State of Missouri.

2.2 Processing Technology

The genetic breed, animal age, feed, curing ingredients, curing procedure, and ham aging environment all contribute to the quality and flavor of dry-cured hams (Ockerman et al., 2002). Many breeds of hogs are used for pork production in the United States, but the most popular breeds for dry-cured ham production include Duroc and Berkshire due to greater amounts of intramuscular marbling, water holding capacity, cathepsin activities and lower pH (Ockerman et al., 2002). Reddish pink, firm and non-exudative (RFN) hams are more desirable for dry-cured ham production than pale, soft, and exudative pork. Most hogs are fed on soybean and corn meal in the U.S while hogs are fed on acorns in the orchards or pastures in Spain (Ockerman et al., 2002).

Dry cured hams are manufactured by salting/curing, smoking (optional) and aging (Graham et al., 2012). The main ingredients in the cure mix are salt, nitrate and/or nitrite. Some processors use sugar and/or a spice blend to enhance the flavor of the ham. Salting can be conducted by immersing each ham with the curing mix or by adding a weighed out amount of curing mix per ham depending on the weight of each ham. The curing agents first penetrate the *semimembranosus* muscle and then slowly diffuse through the rest of the whole ham that is stored at 1-4 °C. The curing time is dependent on type of ham, ham weight and ham processor. In the United States, most processors allow their hams to cure for 40-50 d. Excessive surface salt is then washed off the ham surface with

water and moved to an equalization room that is maintained at 10-12 °C for approximately 15 d. Relative humidity is controlled at or lower than 75% to inhibit microbial growth and the production of unpleasant flavors. Salt penetration lowers the water activity to below 0.92, which preserves the ham (USDA 9 CFR 319.106). Salt also enhances flavor, penetration and moisture reduction. Sugar, on the other hand, can help increase the action of salt, counteract harshness, improve flavor, act as a the substrate for bacteria that transforms nitrate to nitrite as well as keeps the ham more moist and soft during aging (Ockerman et al., 2002). Smoking is another step that some processors in the United States use to further preserve their hams. It gives the ham a darker skin color and smoky flavor as well as extends shelf-life. A cold smoke below 35 °C for roughly 24h with hickory, maple or fruitwoods is normally practiced in the United States.

After curing, hams are then moved to a “summer” room with a temperature of 25-30 °C and 60-80% RH, where the drying/aging stage takes place. Ham aging in the US can be as short as 30 d or up to 2 years. During aging, enzymes including calpains, cathepsins and other enzymes are responsible for extensive proteolysis, which produces free amino acids and small peptides. Leucine, valine, tyrosine, lysine, alanine, glutamic and aspartic acid concentrations increase as dry-cured ham aging time is increased. The combination of these amino acids and small peptides from protein breakdown and proteolysis contributes to the unique flavor of country hams (Toldrá & Flores, 1998). Breakdown of the proteins also softens the texture of the ham. The lipases and phospholipases are responsible for the breakdown of triacylglycerols and phospholipids respectively, triggering the generation of free fatty acids (Toldrá, 2004). These fatty acids are one of the sources for taste and generation of aroma compounds. These mono and

poly-unsaturated fatty acids are susceptible to further oxidation, which contributes to the volatile profile of dry cured ham (e.g. aldehydes, pyrazines, esters, ketones, alcohols and sulfur compounds).

2.3 Ham Mite Infestations on Dry Cured Ham

The environment where dry cured hams are aged is favorable for pest infestations of stored food products by larder beetles, *Dermestes lardarius* L. (Coleoptera: Dermestidae), red-legged ham beetles, *Necrobia rufipes* (F.) (Coleoptera: Cleridae), cheese skippers, *Piophilidae casei* (L.) (Diptera: Piophilidae) and ham mites, *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae) (Graham et al., 2012). Ham mite, *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae), also known as the cheese, mold, copra, and cereal mite is the dominant pest in dry cured pork aging facilities. The mold mite is a universal and synanthropic species that infests various kinds of stored products such as grains, peanuts, medicinal herbs, cheeses, nuts, copra, dried eggs, cottonseed, rapeseed, sunflower seed, dried bananas, wheat spillage, tobacco and flour (Bozcek, 1991). They are often found in stored foods with high concentrations of fat and protein. These products include dried fruits, spices, cultured cheeses (Rentfrow et al., 2008), pet food (Thind, 2005; Brazis et al., 2008), and cereal-based food products (Thind & Clarke, 2001).

A male mite can fertilize as many as 450 females in one life cycle. A female mite lays an average of 4 eggs per day and is capable of laying up to 60 eggs per day. A female mite often produces approximately 500 eggs or more in one life cycle, depending on the environmental conditions. At 60% to 80% relative humidity and 20 to 30 °C, ham mites can complete one generation in 8 to 21 d, and the lifespan increases as the growth

temperature decreases (Mueller et al., 2006). Development does not progress at temperatures below 8.5 or above 36 °C, but eggs can survive for 21 d at -10 °C and 24 d at -5 °C in an inactive state (Boczek, 1991). The optimal temperatures for egg laying are between 22 and 26 °C. Females on yeast or wheat germ can lay eggs within 24 h after mating at 20 °C and 85% relative humidity and produce up to 500 eggs in their lives, with 70% of the eggs laid during the first 3 weeks of their life (Boczek, 1991).

Ham mites are drawn to the aging facilities because dry cured hams go through extensive proteolysis and lipolysis during the aging period. Mite infestations are not likely when their aging time is 3 months or less. However, aging longer than six months is necessary to obtain the desired flavor and product quality for companies that want to meet a niche high dollar market in the United States. The risk of mite infestation increases when ham is aged for more than five months. However, aging for less than five months does not guarantee processors that mite infestation problems will not occur (Rentfrow et al., 2006). Most mite infestations occur on the surface of food products. However, mites can crawl to cracks and crevices and may penetrate inside the product and thus cause more significant economic losses (Zd'árková, 1991). The infested surface sometimes appears to move when observed by the naked eye due to the massive mite population.

Dry-cured ham is a food source for mites since it has sufficient fat and protein for mites and mold to grow on the ham surface. In addition, aging environment conditions in the Southeastern United States provides desirable growth temperatures and relative humidity for mites to thrive and reproduce. Dry cured ham processors in both Spain (Sánchez-Ramos & Castañera, 2000) and the United States (Rentfrow et al., 2008) have

deemed that mite infestations are the most serious pest problem for their aging facilities. Thirty-five dry-cured ham plants in the United States participated in a 2008 survey pertaining to mite prevalence in their plants. Fifteen out of the 19 plants (56%) that age their hams longer than 6 months indicated the presence of mites on their hams. When hams were aged less than 6 months, 10 out of 19 plants reported that mites were present on their hams (Rentfrow et al., 2008). Some of the processors that have excellent sanitation practices reported that they still experience mite infestations. This indicates that mites are a serious problem for most dry-cured ham aging facilities and cannot be controlled by cleaning and sanitation alone.

2.4 Controlling Mite Infestations

2.4.1 Methyl Bromide

Methyl bromide (MeBr, CH_3Br) is a fumigant pesticide that has been used worldwide for processing facilities and storage commodities to control pests in these contexts, and also as a quarantine treatment to prevent the spread of exotic pests across borders since the 1930s (Fields & White, 2002). It is odorless and colorless at concentrations used for fumigation and has a boiling point of 3.6°C . Due to its rapid action and broad spectrum, MeBr has been historically used in agricultural sectors to eliminate insect pests, nematodes, weeds, pathogens, rodents and mites (Fields & White, 2002; Johnson, Walse & Gerik, 2012) without leaving residual MeBr in the products. It is also nonflammable and noncorrosive (Bond, 1984). Methyl bromide kills pests by damaging nerve cell membranes and reacting with the sulfhydryl groups in protein (Fields & White, 2002). In 1992, MeBr was classified as a chemical that contributes to the depletion of stratospheric ozone under the Montreal Protocol. It was scheduled for

developed countries to eliminate bulk use of MeBr by 2005 (Bell et al., 1996). The Montreal Protocol is an international agreement that was ratified by 182 countries, including the United States that pertains to phasing out substances that deplete the ozone layer (Osteen, 2003). Prior to the phase-out, approximately 27,000 metric tons of methyl bromide was used in the United States on an annual basis. The majority of this methyl bromide (75%) was used for soil fumigation, 11% was used for commodity treatments, 6% for structural fumigation, and the remainder was used as feed stock in industrial chemical production (Ragsdale & Vick, 2001).

2.4.2 Recent Methyl Bromide Alternatives Research

Fumigants such as sulfuryl fluoride, phosphine, carbon dioxide, and ozone, food safe compounds, and physical and chemical control methods (heat and cold vs time, pesticides) have been evaluated for their efficacy at controlling mite infestations on whole hams.

2.4.2.1 Sulfuryl fluoride

Sulfuryl fluoride was developed in the 1950s (Meikle & Stewart, 1962) and is currently produced by Douglas Products (Liberty, MO) under the trade name ProFume. It is an alternative to methyl bromide for some applications and was registered for use in dry cured ham in the summer of 2005 (EPA, 2005). Research indicated that sulfuryl fluoride was effective at controlling all life stages of red-legged beetles at the label rate of 36 mg/L (Phillips et al., 2008). However, the mortality of ham mites required much greater concentrations of SF, with three times the legal label rate of sulfuryl fluoride applied causing 95% mortality of ham mites. Furthermore, studies by Sekhon et al.

(2010b) implied that the level of residual fluoride ion was linearly related to sulfuryl fluoride fumigation concentration and that increasing fumigation times and greater concentrations of sulfuryl fluoride may lead to residual sulfuryl fluoride and fluoride ion accumulating in the ham at levels greater than the EPA (2005) limits of 20 ppm fluoride ion and 0.01 ppm sulfuryl fluoride. Based on these results, it was concluded that sulfuryl fluoride is not a viable alternative to methyl bromide for controlling ham mites.

2.4.2.2 Carbon dioxide

Hasan et al. (2016) evaluated CO₂ for its efficacy at controlling *Tyrophagus putrescentiae*, ham mites and *Necrobia rufipes*, red-legged ham beetle infestations. Results indicated that 144 h of exposure to 80% CO₂ was necessary for 100% mortality of all life stages of red legged ham beetles and mites (Sekhon et al., 2009a; Sekhon et al., 2010c; Hasan et al., 2016). This treatment would not be practical since ham facility structures are not air-tight and the exposure time is too long of a period to treat the hams since the plants would incur economic losses if they were closed for 6 d at a time.

2.4.2.3 Phosphine

Researchers reported that fumigation with 400 ppm PH₃ caused 100% mortality of all life stages of red-legged ham beetles and fumigation at 1000 ppm PH₃ caused 100% mortality of ham mites (Phillips et al., 2012). Further studies confirmed that residual concentrations of PH₃ in dry cured hams were below the legal residual limit in stored food products (0.01 ppm) that were fumigated with 1000 ppm PH₃ for 48 h (Sekhon et al., 2009b; Sekhon et al., 2010a). In addition, consumer panelists were not able to differentiate between control and PH₃ fumigated ham slices (Sekhon et al., 2009b;

Sekhon et al., 2010a). Zhao et al. (2015) conducted three fumigation trials: one under lab conditions, one in simulated aging houses and, one in a commercial processing plant trial to evaluate mite and red-legged beetle mortality, PH_3 residue, and sensory effects due to fumigation. These researchers concluded that PH_3 was successful at controlling mites under laboratory conditions. However, phosphine corroded the electrical system of the ham aging facilities in a plant trial, which indicates that it would be challenging to use in dry-cured ham processing plants.

2.4.2.4 Ozone

In a benchtop study, concentrations of ozone greater than 150 ppm with 48 h exposure time caused 100% mortality of red legged ham beetles and ham mites (Sekhon et al., 2010c; Hasan et al., 2016). However, due to ozone's lack of ability to penetrate surfaces and the ability of mites to hide in places where ozone cannot reach, it would not be effective at controlling mites under real world conditions.

2.4.2.5 Food safe compounds

Abbar et al. (2016b) evaluated the efficacy of food safe chemicals on controlling mite growth on dry-cured hams. These compounds included salts and free acids (calcium propionate, sodium propionate, calcium sorbate, sodium sorbate, potassium iodate, citric acid, etc.), oils/fat (canola, light mineral, soybean, olive and lard), organic alcohols (1-Propanol, 2-propanol, 1,2- propanediol, 1,3-propanediol, 1,2-butanediol, 1,3-butanediol, glycerol) and other additives (carrageenan, propylene glycol alginate, xanthan gum, butylated hydroxyanisole, butylated hydroxytoluene, natamycin, ethoxyquin). Corn oil, olive oil, calcium citrate, potassium citrate, sodium citrate, potassium propionate,

potassium sorbate, sodium carbonate and natamycin did not control mite growth in comparison to the controls. Meanwhile, different concentrations of salts and free acid treated ham cubes had significantly lower mite reproduction after 2 weeks of incubation. For example, calcium sorbate at 10% had a 4-fold growth reduction on the cubes in comparison to the control cubes. Oils and fat (vegetable and non-vegetable oil-lard) also caused growth reduction on the treated cubes. Propylene glycol (1,2-propanediol) and lard controlled mites at concentrations at 100%. Based on these results, Zhao et al. (2016a) conducted experiments on the efficacy of using PG, lard, and the combination of PG and gums to control mite infestations.

2.4.2.6 Insecticides

Abbar et al. (2017) evaluated different registered pesticides, including azadirachtin, rosemary oil, Avermetin, Chlorphenapyr, Tau-fluvalinate, deltamethrin, gamma-cyhalothrin, deltamethrin + chlorpyrifos methyl, Malathion, Carabryl and Bifenazate for their efficacy at controlling *T. putrescentiae* through mite bioassays in the laboratory. Results indicated that deltamethrin plus chlorpyrifos-methyl, chlorphenapyr and malathion increased mite mortality. In addition, chlorphenapyr was effective at controlling ham mites when applied to wood, concrete and metal for 8 weeks. Therefore, chlorphenapyr could be used on non-food contact surfaces as part of an integrated pest management program to control mites.

2.4.2.7 Temperature control vs time

Temperature control studies were conducted by Abbar et al. (2016a). At -7 °C, 100% mortality of adults and nymphs was achieved after 12 h of exposure, but mortality

of eggs didn't reach 100% even after 48 h of exposure. Adults, nymphs and eggs did not survive after 18 h at -10 °C. This indicated that freezing at -10 °C for 18 h of exposure may be successful at controlling mites. One hundred percent mortality of nymphs was achieved at 40 °C for 48 h while eggs took 72 h or longer. At 42 °C, adults, nymphs, and eggs were controlled after 12 h of exposure. These results indicate that exposing infested hams to 42 °C for 12 h may be an effective way to control mites (Abbar et al., 2016a). These temperature controls would need to be applied to commercial plants or simulated aging houses to evaluate sensory quality and confirm that these conditions will control mites when applied in commercial aging rooms.

2.5 Food Grade Coatings

2.5.1 Introduction

Edible films and coatings can be applied on food surfaces to control gas exchange, moisture permeability or oxidation and to extend the shelf-life of foods, such as fruits, vegetables, and meat products. The history of using films on foods traces back to the 12th century in China where waxes were utilized to coat citrus fruits to reduce water loss. The first edible films for food preservation were extracted in the 15th century from soymilk in Japan (Sánchez-Ortega et al., 2014). The first edible film that was used as a coating on meat was lard that was used to extend the shelf-life of meat products in the 16th century in Europe. This process is referred to as “larding” (Cagri et al., 2004; Pavlath & Orts, 2009). Various types of edible coatings have been developed for food applications.

A wide range of lipid compounds have been used in edible films and coatings, such as animal and vegetable oils and fats, waxes, natural resins, essential oils and

extracts, emulsifiers, and surface active agents. These lipids are added to improve hydrophobicity, cohesiveness, and flexibility, as a barrier to moisture transfer, and to prolong color, aroma, freshness, tenderness, and microbiological stability (Debeaufort & Voilley, 2009; Genneadios et al., 1994; Genneadios et al., 1997).

Protein films are mostly derived from animal proteins (collagen, gelatin, casein, whey protein concentrate and isolate and egg albumin) or plant proteins (corn zein, soybean protein, wheat gluten, cottonseed, and peanut proteins etc) (Gennadios et al., 1994; Torres, 1994). Protein films have a relatively higher water vapor permeability than conventional polymeric packaging materials due to the hydrophilic nature of proteins and plasticizers.

A variety of polysaccharide-based films and coatings have been studied and/or used in food applications, such as cellulose, starch (native and modified), pectins, seaweed extracts (carrageenan, alginates, and agar), gums (acacia, tragacanth, and guar), pullulan and chitosan. These polysaccharides can be used to prolong the shelf-life of meat and meat products by preventing dehydration, oxidative rancidity and surface browning (Sánchez-Ortega et al., 2014). Several active ingredients can be incorporated into the polymer matrix of the coating to improve safety, nutritional and/or sensory attributes (Rojas-Graü et al., 2009).

Antimicrobial compounds have been incorporated into edible films and coatings as an alternative to direct applications onto the surface of the meat in order to release the antimicrobial compound gradually and reduce the amount of antimicrobial that is used and reduce the impact of the antimicrobial on the sensory characteristics of the product (Sánchez-Ortega et al., 2014).

Films made from high methoxyl pectin + apple, carrot or hibiscus puree with 3% carvacrol reduced *Listeria monocytogenes* by 3 logs on ham when compared to the control after 7 d of storage and 2 logs on bologna after 7 d of storage (Ravishankar et al., 2012). Hong et al. (2009) used 0.08% of grapefruit seed extract or 2.8% green tea extract in *Gelidium corneum*-gelatin films and demonstrated a decrease in *Escherichia coli* and *Listeria monocytogenes* population by 1 and 2 log CFU/g on pork loins, respectively, in comparison to the control. Shelf life of chicken breast fillets was extended by 6 d or greater with chitosan (1.5%) coating and/or oregano oil (0.25%) (Petrou et al., 2012). These research efforts indicate that using edible films and coatings was an efficient method to deliver the active ingredient(s) (antimicrobial compounds) to reduce bacterial counts in meat and meat products.

2.5.2 Development History of Coatings for Dry-cured Ham

Abbar et al. (2016b) conducted mite reproduction assays by dipping ham cubes in various food compounds and inoculated the cubes with mites to evaluate their efficacy at controlling mites. Results indicated that 50% 1, 2-propanediol (propylene glycol) and 100% lard were effective at controlling mite growth under laboratory conditions. Most edible films and coatings that are used for foods are impermeable to gas and moisture. However, dry-cured ham is required by law to lose at least 18% of its weight from the original weight of the ham (USDA 9 CFR 319.106). Since lard is impermeable to moisture and oxygen, different food grade films and coatings that were incorporated with or without propylene glycol and glycerin were evaluated for their efficacy at controlling mite reproduction on ham cubes (Zhao et al., 2016a). Xanthan gum (XG, 1%) and a combination of propylene glycol alginate (PGA, 1%) and carrageenan (CG, 1%) were

able to reduce mite growth on ham cubes by over half in comparison to control cubes. When XG was combined with at least 20% PG and PGA+CG was combined with at least 10% PG respectively, mite growth was completely inhibited with either zero or 2 mites on the ham cubes (Zhao et al., 2016a). To be able to apply these coatings on dry-cured ham, the coating needs to be moisture and oxygen permeable as well as form consistent films that are thin and flexible on the surface of the dry-cured ham. Zhao et al. (2016a) tested the thickness and water vapor permeability as well as oxygen transmission rate. Results indicated that when PG concentrations increased, the thickness and water vapor permeability increased and the oxygen transmission rate was limited by these coatings.

XG, PGA, CG and PG are generally recognized as safe (GRAS) and commonly used in multiple foods as additives. Xanthan gum is used in sauces, frostings, fruit gels, and gravies (Nisperos-Carriedo, 1994) and in salad dressing with propylene glycol alginate (Pettitt et al., 1995). Carrageenan is approved as a direct food additive as an emulsifier, stabilizer and thickener (Baldwin, 2007). Alginates are derived from brown seaweed and have been widely used in edible films and coatings. King (1983) stated that coatings made by evaporating water from a thin layer of alginate solution are impermeable to oils and greases, but, when combined with other hydrophilic polysaccharides (such as carrageenan) exhibit high water vapor permeability (King, 1983). Propylene glycol is GRAS and used in the food industry for multiple purposes such as an anticaking agent, antioxidant, flavor agent, and emulsifier (FDA 21 CFR 184.1666).

XG, PGA + CG and PG combinations were used for mite residency tests on whole hams by inoculating approximately 900 mites per ham (Abbar et al., 2016b).

Results demonstrated that hams that were coated with PGA + CG + 20% or 40% PG had fewer than 10 mites on the whole hams after 6 weeks of aging (Abbar et al., 2016b). This indicated that ham mites did not reproduce on the treated hams. The inhibitory effects of PG to ham mites is not clear, but, propylene glycol has demonstrated antimicrobial properties against *Candida albicans*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus mitis* and *E. coli* with 20 h of exposure (Kinnunen & Koskela, 1991). Thus, these food grade coatings demonstrated potential as an alternative for methyl bromide. However, further PG concentrations below 20% can still be investigated to make it more economically feasible for processors to use this technology.

2.6 Ham Nets as Coating Carrier

2.6.1 Bioactive Fibers to Control House Dust Mite Complex

T. putrescentiae is a source of allergen and is one of the mites associated with the house dust mite complex. Due to the tiny nubs of 10-40 microns of its excrement, which contains antigens that may induce an allergic reaction when inhaled (Niekraszewicz et al., 2005), the house dust mite complex is the most common inhalatory allergen (Fain et al., 1990). Most patients who have bronchitis, rhinitis and dermatitis have a high risk of reacting to house dust (Kowalski, 2000; Van Bronswijk & Schober, 1991). Thus, the textile industries have developed barrier textiles to prevent mite growth such as coverlets, mattresses and pillowcases that are impenetrable to mites, their excrements and allergenic particles (Brzeziński et al., 1996). These textiles were not sufficient to protect against house dust mites. Mites do not populate on the medium of exfoliated skin cells (too dry and fatty). However, *Aspergillus repens* can degrade the skin so that it can be utilized by

the mites as food sources (Service, 1998; McClellan et al., 2003; Cox et al., 1998). Companies in different countries have manufactured products with fibers that use a biocidal agent to control *A. repens* growth and therefore inhibit the growth of house dust mites. For example, a company from the United Kingdom has produced an anti-microbial, polyacrylonitrile fiber and an anti-mite fiber (Jackowski et al., 2004). The French company, Rhovyl has manufactured a polyvinylchloride anti-mite fiber, which contains benzyl benzoate (a medication and insect repellent on skin) (Bohringer et al., 2000). The Austrian company, Asota GmbH, has manufactured an anti-mite fiber (Asota AM Plus) that contains biocides with a trade-name of MB E 97-65 (Schobesberger, 1998). Nishioka et al. (1997) investigated a bedding encasement of mite blocking fibers on infants with atopic dermatitis and concluded that the bedding encasement was effective at preventing atopic infants from being sensitized to house dust mites. Anti-mite modified spun polypropylene fibers (with ceramite) and bedding inserts containing such fibers were evaluated for their anti-mite action and were able to mitigate allergic reactions by patients (Niekraszewicz et al., 2005). Borkow and Gabbay (2004) and others incorporated copper into textiles and fabrics to produce anti-viral masks, gloves and filters to protect from HIV-1, flu and other viruses, and anti-bacterial fabrics to destroy antibiotic resistant bacteria (Gabbay et al., 2006). In addition, anti-fungal socks have been developed to treat athlete's foot (Zatcoff et al., 2008). Tightly woven and plastic covers have been used as bed encasings to prevent mite penetration, while non-woven, loosely woven, acaricide-coated or laminate covers did not inhibit mite penetration (Mahakittikun et al., 2006). Tightly woven covers performed better than the other materials with greater than 99% allergen impenetrability, resistance to live mite

penetration, dust leakage of less than 4%, air permeability between 2 and 6 cm³/sec/cm², a thread count $\geq 246/\text{in}^2$, and a pore size of 2–10 μm (Mahakittikun et al., 2009).

2.6.2 Meat Encasement-Stockinettes

The first documented use of stockinettes to hang and/or smoke ham was in 1919 by the Department of the Interior Bureau of Education in the United States (Claxton, 1919). Rumsey and Netley (1941) developed porous stockinettes as casings for pork butts to produce the desired surface appearance and facilitate smoke penetration. Encasing meat and poultry products, especially during the cooking and/or smoking process, is commonly done for hams and poultry. The netting encasements usually consist of an arrangement of equally sized squares made of polyester or cotton and an elasticized strand material in a tubular form. The elasticized material creates tension and forms squared indentations on the outer surface of the meat product, which leaves the desired “checkerboard” pattern on the surface of the meat. Use of collagen films with a netting arrangement is a common practice to enhance the appearance of meat products (Mintz, 1995). However, collagen films are expensive. Thus stockinettes were developed to replace collagen films (Mintz, 1995). Stockinettes are made from cotton, polyester, nylon or other suitable materials. Since stockinettes are knit or woven, they have openings and are stretchable (Mintz, 1995). Most ham stockinettes are made of spun cotton, poly-cotton, polyester or acrylic yarns and are either jersey or rib knit fabrics. The gauge strength of the net fabrics is dependent on the stitch density (Elsasser, 2005a). Higher stitch density indicates finer fabric and smaller mesh size.

Stockinettes are sometimes treated with liquid smoke, oils, or acid solutions and may be coated with cellulose to enhance the peelability of the nets or the color and/or

flavor of the meat product. The textile industry often uses a padding machine to apply both chemical and additive finishes, either in liquid or paste form, by immersing or transferring from a roller (Hollen & Saddler, 1955). The “padding” is accomplished by feeding the fabric through the finishing solution under a guiding roller and between two padding rollers, which are either made of metal or rubber depending on the finish solution. The pressure exerted from the rollers squeezes the solution into the fiber or fabric, while squeezing excess solution out of the net (Hollen & Saddler, 1955).

2.6.3 Potential Bioactive Stockinette for Dry-cured Ham

Most dry-cured hams in the United States are placed in a stockinette and hung on a rack during aging. However, these are often inexpensive polyester nets that are usually not treated with any antimicrobial since the high salt content inhibits bacterial growth on the ham.

2.7 Sensory Evaluation

Several factors affect the meat flavor quality of dry-cured hams, including animal age, genetic breed, nutrition, pre-slaughter environment conditions, etc., but postmortem processing is the main factor that affects the final product quality (Spanier et al., 1990). Even though the coatings that have been developed are food-grade and applied prior to aging, it is important to evaluate if these coatings and netting treatments would affect sensory properties of the dry-cured ham. Dry-cured hams that were fumigated with phosphine (0, 200 and 1000 ppm for 48 h) and methyl bromide (0, 4, 8,16, and 32 mg/L for 48 h) were evaluated by consumers using triangle tests. In these tests consumers did not detect any differences between the control hams and both the phosphine and methyl

bromide fumigated hams (Sekhon et al., 2010a). In another study, the effect of sulfur dioxide (0, 12, 24, 36 and 72 mg/L) fumigation on the sensory quality of dry-cured hams was evaluated by consumers in triangle tests, and no difference existed between the control and the hams that were fumigated with sulfur dioxide at 0 and 36 mg/ml (Sekhon et al., 2010b). Drying of hams in a reduced oxygen atmosphere ($O_2 < 4.5\%$) inhibited mite growth. However, it caused a negative impact on the sensory attributes and was, therefore, considered inappropriate for use in producing traditional dry-cured hams (Sánchez-Molinero et al., 2010; Sánchez-Molinero & Arnau, 2010). Pham et al. (2008) determined that the relationship between sensory descriptors, consumer acceptability and volatile flavor compounds for 8 commercial American dry-cured hams using external preference and flavor mapping. Dry-cured ham products with more intense caramelized, smoky, savory and molasses aromas as well as more intense sweet and savory flavors received higher consumer acceptability scores. Difference from control tests were used to evaluate hams that had been fumigated with phosphine in commercial ham aging houses and simulated aging houses. In this testing, there were no detectable differences between the control hams and phosphine fumigated hams (Zhao et al., 2015). In research conducted by Zhao et al. (2016a), ham slices that were treated with food grade coatings did not differ from the control hams with respect to sensory characteristics (Zhao et al., 2016a). Dry-cured hams made without nitrite and cured at low temperature (3-4 °C) were investigated with respect to red color development. Results indicated that red color was formed at low temperature, but at a slower rate and with a less intense color than at warmer conditions (13-15 °C) (Porolari et al., 2016). Color and descriptive sensory analysis both indicated that a non-enzymatic mechanism led to the formation of Zn

protoporphyrin (the red color) at 3-4°C with an approximate three-month delay in comparison to nitrite-free hams produced at 13-15 °C (Poralari et al., 2016).

Sensory evaluation is defined as “a scientific discipline used to evoke, measure, analyze, and interpret those responses to products that are perceived by the senses of sight, smell, touch, taste, and hearing” (Stone & Sidel, 1993). There are two groups of difference tests: overall difference tests and attribute difference tests. Overall difference tests are designed to evaluate if noticeable differences exist between samples. Triangle, Duo-trio, two out of five, simple difference, “A”-“Not A”, difference from control, and sequential tests are examples of overall difference tests (Meilgaard et al., 2007). Attribute difference tests are used to determine if and how a certain attribute differs among samples.

The objective of difference from control tests is twofold: 1) The first objective is to determine if there is a difference between the control and one or more samples; 2) The second objective is to estimate the magnitude of difference between the control and one or more test samples (Meilgaard et al., 2007). A labelled control sample and one or more test samples are provided to each panelist. Panelists should be notified that some of the test samples may be the same as the control. The mean difference-from-control estimates are evaluated by comparing them to the difference-from-control ratings that are obtained for the blind controls (Meilgaard et al., 2007). When running difference from control tests, panelists should either be untrained or trained, but not a mixture of the two. All panelists need to be familiar with the meaning of the scale, the test format and the fact that a proportion of test samples are blind controls (Meilgaard et al., 2007).

Difference-from control tests are limited because the differences usually are not defined or clear. Therefore, descriptive analysis is often used to define the differences that are detected. Descriptive analysis methods are used to detect/discriminate and describe both the qualitative and quantitative sensory properties of a product by trained panelists. Panelists should be trained to understand the definitions of the sensory descriptors. Sensory attributes and the definition of these attributes should be selected based on the real chemical and physical properties of the samples that are perceived (Meilgaard et al., 2007). Qualitative characteristics include appearance (color, surface texture, size, shape, interactions among pieces or articles), aroma (olfactory sensations, nasal feeling factors), flavor (olfactory sensation, taste sensations, oral feeling factors), oral texture (mechanical parameters such as hardness and viscosity, geometrical parameters such as gritty, grainy, flaky, fat/moisture parameters such as oily, juicy, wet), and skin feel (mechanical, geometrical fat/moisture and appearance parameters such as thickness, foamy, gloss, greasy) (Meilgaard et al., 2007). The sensory descriptors for dry-cured hams include rancid, molasses, fermented, caramelized, pork complex, smoky, earthy, savory for aroma; cured, rancid, salty, aftertaste, pork complex, sweet, savory, bitter, astringent, mouth-drying and salt-burn for flavor; hardness, dryness, fibrousness, juiciness, chewiness, mushiness for texture; color homogeneity and marbling for appearance (Armero et al., 1999; Ruiz et al., 1998; Pham et al., 2008). The quantitative factor of descriptive analysis is the magnitude of the descriptor that is present on a 15-cm line scale, where zero indicates that the descriptor is not detectable and 15 indicates the maximum intensity for the descriptor (Civille, 1979). Descriptive analysis methods include the flavor profile method, texture profile method, quantitative descriptive analysis

(QDA®) method and Spectrum™. The flavor profile method includes analyzing the aroma and flavor characteristics that are perceived and their intensities, order of appearance and aftertastes by the panelists. The texture profile method was developed to define the textural parameters of foods. The QDA® method selects panelists based on their capability of discriminating differences in sensory properties among samples of a specific product class. The Spectrum descriptive analysis method is a “custom design” approach to panel development, panelist selection and training, and maintenance.

Difference from control tests can be conducted to evaluate if trained panelists can detect a difference between treated and control hams, when dry-cured hams are either treated with food grade coatings or nets that are infused with such coatings. If there are differences detected, descriptive analysis can be carried out to define and describe these differences. Consumer acceptability tests could then be utilized to determine if consumers dislike the changes that are imparted by the treatments.

CHAPTER III

MITE CONTROL AND SENSORY EVALUATIONS OF DRY-CURED HAMS WITH FOOD-GRADE COATINGS

3.1 Introduction

Dry-cured hams, referred to as southern country hams in the United States, are produced from the hind leg of a hog and cured by rubbing a dry salt curing mix on the surface of the hams, followed by salt equalization and aging (Marriott & Ockerman, 2004; Zhao et al., 2016b). Unique characteristic flavors and aromas are developed during aging due to extensive lipolysis and proteolysis (Toldrá & Flores, 1998). The amount of time that hams are aged varies from 3 to 36 months depending on the aging condition and the region (Toldrá & Aristoy, 2010). The ham mite, *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae), also known as the mold, cheese or copra mite infests stored food products such as grains, whole wheat flour, soy flour, peanuts, cheese, nuts, copra (dried coconut), dried eggs, bacon and dry-cured hams (Hughes, 1976; Van Hage-Hamstem & Johansson, 1992). Due to the high fat and protein composition, water activity and moldy surface, dry-cured hams have a high susceptibility to mite infestations starting at 4-6 months into the aging process (García, 2004; Rentfrow et al., 2008). Dry-cured hams are aged in environments that facilitate mite reproduction and population growth (Sánchez-Ramos & Castañera, 2000; Rentfrow et al., 2012). The optimal growth conditions for ham mites include 23.2 ± 2.1 °C and $71 \pm 5.6\%$ RH (Sánchez-Ramos & Castañera, 2005; Sánchez-Ramos et al., 2007; Aspaly et al., 2007; Qu et al., 2015), which are similar to the temperatures and relative humidities in dry-cured ham aging houses.

Methyl bromide is a fumigant pesticide that has been used globally to control pests in stored commodities and processing facilities (Fields & White, 2002). It also has been used as a quarantine treatment to prevent the movement of exotic pests across borders since the 1930s (Fields & White, 2002). Methyl bromide is a stratospheric ozone layer depleting substance (Marriott & Schilling, 2004) and is being phased out of all industries by the United Nations through the Montreal Protocol, an international agreement ratified by more than 180 countries (Fields & White, 2002). As of 2008, 22 out of 35 dry-cured ham plants in the United States used methyl bromide fumigation to control ham mites (Rentfrow et al., 2008). The only 2016 critical use exemptions for methyl bromide by the U.S. Environment Protection Agency were the California strawberry fruit growers and dry-cured pork producers (EPA, 2015b). However, it was determined in 2015 that methyl bromide stocks are available for use by the U.S. dry-cured pork industry and therefore there is not currently a need for a critical use exemption until existing stocks are depleted (EPA, 2015a).

Food grade coatings and edible films have been used on candies, fresh fruits, vegetables and processed meat products to enhance appearance, texture, stability or quality and reduce water loss (Baldwin, 2007). Food grade coatings made with propylene glycol alginate, carrageenan, xanthan gum, water, and propylene glycol as the active ingredient were previously effective at controlling mites on ham cubes (Zhao et al., 2016a; Abbar et al., 2016b). Propylene glycol alginate (FDA 21CFR172.858), carrageenan (FDA 21CFR172.620), xanthan gum (FDA 21CFR172.695) and propylene glycol (FDA 21CFR184.1666) are generally recognized as safe (GRAS) compounds. The minimum effective concentration for propylene glycol was 10% for propylene glycol

alginate and carrageenan and 20% for xanthan gum (Zhao et al., 2016a). Similar coatings with 20 and 40% propylene glycol significantly reduced mite colonization and residency on treated whole hams (Abbar et al., 2016b). If the propylene glycol concentration in these coatings could be decreased further, it would substantially reduce coating costs. However, no results have been reported on whether these coatings cause a perceivable sensory difference between dry-cured hams that are not treated with coatings. Therefore, the first objective of this paper was to determine the lowest concentration of propylene glycol that controls mite growth on dry-cured ham cubes; the second objective was to apply the coatings in commercial ham aging facilities and evaluate the sensory differences between hams that were treated with coatings prior to aging and non-treated control hams by utilizing difference from control sensory tests.

3.2 Materials and Methods

3.2.1 Coating Composition Optimization

3.2.1.1 Materials

Propylene glycol (Essential Depot, Sebring, FL) was included at concentrations of 0, 10%, 15%, and 20% in coatings made with 1% xanthan gum (TIC Gums, White Marsh, MD) in water. In addition, propylene glycol concentrations of 0, 2.5%, 5%, 7.5% and 10% were used in coatings made with 1% propylene glycol alginate (TIC Gums, White Marsh, MD) and 1% carrageenan (TIC Gums, White Marsh, MD). A coating with 10% propylene glycol with 0.5% propylene glycol alginate and 0.5% carrageenan was also evaluated to determine if a lower concentration of gum could be used in the formulation.

3.2.1.2 Ham cube preparation

Dry-cured hams that had been aged for approximately 90 d, weighing approximately 8 kg each, were purchased from a commercial dry-cured ham plant. Ham slices (2.5 cm thickness) were cut from each ham, and slices were cut into $2.5 \times 2.5 \times 2.5$ cm³ cubes. Xanthan gum coatings were solubilized at room temperature, PGA + CG coatings were solubilized with boiling water using a hot stir plate and then cooled to between 28 and 30 °C (Zhao et al., 2016a). Ham cubes (n = 5) were randomly selected and dipped directly into each treatment of the food grade coatings for 10 seconds with a cotton string and allowed to drip for one min to dry prior to wrapping in wax paper (Reynolds, Richmond, VA) and packaging in zip-loc bags (Ziploc, Racine, WI). Bags were then packaged with icepacks and shipped overnight to Kansas State University, Manhattan, KS, and mite reproduction assays were conducted.

3.2.1.3 Mite reproduction assay

Mites were from a laboratory colony at Kansas State University that were reared using the methods described by Abbar et al. (2016b). Twenty mixed sex adult *T. putrescentiae* (2 to 3 weeks old from culture) with an average of 10-12 females were inoculated onto each cube in a randomized order. Each cube was placed in a glass mason jar (216 ml, 65mm diameter, 55 mm height; Ball Corp., Broomfield, CO) and incubated at 25 ± 1 °C and 70% relative humidity for 14 d. Resulting populations of mobile adult and immature mites on the ham cubes were counted using a dissecting stereo-microscope (Olympus Model SZX10, Olympus Surgical & Industrial America INC) in a randomized order after 2 weeks of incubation to determine how well coatings inhibited the 20 initial mites from reproducing.

3.2.2 Application of Food Grade Coatings to Whole Dry-Cured Hams

3.2.2.1 Materials

Food grade coatings that were developed by Zhao et al. (2016a) were used to dip whole hams prior to aging. For the first trial, whole hams from each commercial plant were dipped in either xanthan gum only, propylene glycol alginate + carrageenan only (PGA + CG only), xanthan + 20% propylene glycol (XG + 20% PG), propylene glycol alginate + carrageenan + 20% propylene glycol (PGA + CG + 20% PG), propylene glycol alginate + carrageenan + 40% propylene glycol (PGA + CG + 40% PG) or PGA + CG + 20% PG net only (net only). For the “net only” treatment, only the nets used by the processors were dipped in PGA + CG + 20% PG coating solution instead of the whole hams. XG, PGA and CG were used at 1% and control hams were not dipped and placed next to the coating dipped hams. Xanthan gum coatings were solubilized in room temperature water. PGA + CG coatings were solubilized in PG and water was added into the mixture as the solution was heated to a boil. PGA + CG based coatings were then cooled to 30-35 °C (Zhao et al., 2016a). Greater concentrations of PG were used in both plant trials in comparison to concentrations that were used in laboratory testing. We presumed that if high concentrations of PG did not cause noticeable consumer sensory differences between control and treatment hams, then it is unlikely that lower concentrations would cause noticeable sensory differences. The first trial was conducted in the summer of 2014 in three commercial processing facilities in Tennessee and Virginia and in a simulated aging house at Mississippi State University for a total of 4 locations. In this initial trial, whole hams were dipped in coatings and aged for approximately 6 months prior to sensory evaluation.

In order to reduce coating application cost and reduce sensory differences between hams, a paint gun with a high pressure spray nozzle (Wagner Flexio 590, Plymouth, MN) was used in the second trial to spray the coatings onto the whole hams. One liter of coating was used to coat two hams for each treatment. Based on the results from the first trial, the treatments in the second trial included the control, PGA + CG only, PGA + CG + 10% PG, PGA + CG + 20% PG, and XG + 20% PG. The second trial was conducted in the summer of 2015 in three different processing facilities in Tennessee and Virginia and the simulated aging house at Mississippi State University for a total of 4 locations. PGA + CG + 20% PG and XG + 20% PG treated hams were evaluated for sensory differences, since greater concentrations of PG would potentially have a greater impact on sensory properties than lower concentrations, if any differences existed.

3.2.2.2 Whole hams and aging

The dry-cured hams that were used had finished the salting and equalization steps and were ready to be placed in the aging house. The hams were treated with coatings and then placed in the aging house with the other commercial hams that were produced that day. The aging environment varied within each processing facility with aging temperatures and relative humidities between 24-28 °C and 60-80% RH, respectively. The breeds of hogs from which hams were processed in each plant were different according to the processors, which included the breeds of Berkshire, Gloucester Old Spots, Red Wattle, Tamworth, Yorkshire, Hampshire and Duroc cross. Whole hams were aged for approximately 6 months. After aging, each facility sent hams back to Mississippi State University for sensory evaluation. In addition, the weight of the hams in the second

trial was recorded for moisture loss to verify that hams were losing enough moisture for the hams to be preserved properly and legal for commerce as per USDA regulations.

3.2.2.3 Sensory evaluation-difference from control test

Difference from control tests were performed to determine if trained panelists could perceive a difference between control ham samples and coating-treated samples. Institutional Review Board (IRB) protocol number 11-230 was approved on 23 August 2011 for “Sensory quality and consumer acceptability of dry-cured ham exposed to processing aides designed to combat pest infestations”. A continuous IRB protocol number 15-246 was approved on 29 July 2015 through 31 August 2018 for “Sensory quality and consumer acceptability of dry-cured ham exposed to food grade coatings, lactic acid fermentation, and other food safe methods for controlling pest infestations”. Coatings on hams were washed off with tap water (20 °C) at room temperature prior to slicing. Hams were sliced (1.3 cm thickness) in the meat laboratory at Mississippi State University using a band saw (Butcher Boy, Lasar MFG. Company, Inc. Los Angeles, CA, USA). Slices were then vacuum packaged into vacuum bags (standard barrier, PVdC, 36 cm × 51 cm, WVTR ≈ 0.4 g/100 in² /24 Hrs, Curwood, Inc, New London, WI) with a dual-chamber ULTRAVAC vacuum packaging machine (Model UV2100, Koch Equipment, Kansas City, MO) at vacuum level of 99% and stored for 1-2 weeks at 0-4 °C prior to cooking. Refrigerated ham slices were equilibrated to room temperature prior to baking. Each ham slice was wrapped in aluminum foil and oven-baked at 177 °C to an internal temperature of 71 °C according to traditional cooking methods by Marriott and Ockerman (2004). The internal temperature was checked using an infrared thermometer

(Horiba IT-330, Horiba Inc, Irvine, CA). Each ham slice was cut into square pieces with similar sizes (1.3 cm × 1.3 cm) from the same muscle (Figure 3.1). Sensory sampling was mainly from muscle section 1, and two pieces were from section 2 or 3 when needed (Figure 3.1). Upon serving, ham pieces were placed into 29.5 ml clear plastic containers (Sweetheart Cup Co., Owing Mills, MD) that were coded with 3-digit random numbers. Each panelist was served samples from the same location on the same muscle for each treatment to avoid sensory variability between muscles. Panelists were trained for two weeks with 6 sessions and 3-5 samples of coated hams and control hams per session to evaluate overall differences in flavor, texture and moistness by two faculty members with experience conducting descriptive panels on dry-cured ham (Pham et al., 2008). A labeled control sample was provided as reference along with the treated samples. A blind control with a 3-digit random number was included in each test as a baseline to account for natural random variation between samples. Trained panelists (n = 6-10, 12 panels per trial, an average of 100 overall ratings for each treatment for each descriptor), each with greater than 30 h of experience in tasting dry-cured ham, were asked to taste the labeled control first and then evaluate samples in a randomized order with 2 or 3 coated hams and blind control hams to rate how different the treatment samples were from the control with respect to flavor, texture and moistness in 3 sessions each week. Water, apple juice, unsalted crackers, napkins, forks, and expectorant cups were provided to the panelists who were seated in separate booths during each panel. Panelists cleansed their palate with unsalted crackers, apple juice and water during a mandatory 20s break between each sample. The scale for the difference from control test was: 1 = no difference, 2 = slight

difference, 3 = moderate difference, 4 = large difference, 5 = very large difference (Meilgaard et al., 2007).

3.2.3 Prices of the Coatings

Ingredient prices were provided by the supplier source based on the market in 2015. The prices of coatings (500 mL) for one ham were calculated based on these information. However, our research team was asked not to disclose the ingredient price information. There was only one source of price for each ingredient in the formulation, thus, no statistical analysis was needed.

3.2.4 Statistical Analysis

A completely randomized design with five replications of each treatment (each cube as an experimental unit, E.U.) was used to determine the effectiveness of PG concentrations in the coatings on controlling mite population growth on treated ham cubes. A randomized complete block design with location serving as a block was utilized for the two commercial trials to evaluate if trained panelists (n = 6-10, 12 panels per trial, an average of 100 overall ratings for each treatment for each descriptor) could detect a difference between coated and non-coated ham samples ($P < 0.05$). A randomized complete block design with location serving as a block was used for the weight loss of hams in the second trial. Statistical analyses were conducted using Compusense (Compusense 5.2 and Compusense Cloud, Guelph, CA) for collecting data and SAS statistical software (SAS 9.4, 2013, SAS Inc., Cary, NC). Proc GLM was used to compare response variables among the different treatments. When differences ($P < 0.05$)

occurred among treatments, Tukey's Honestly Significance Difference Test ($P < 0.05$) was used to separate treatment means.

3.3 Results and Discussion

3.3.1 Mite Reproduction Assay

No difference existed ($P > 0.05$) in the number of surviving mites between the control and 1% (PGA + CG) coating without PG added (Table 3.1). Adding 2.5% PG to PGA + CG resulted in fewer mites ($P < 0.05$) than the control and 1% (PGA + CG) treatments (Table 3.1). As PG concentration increased to 7.5%, mite counts decreased ($P < 0.05$). No difference existed in number of mites among the 7.5% PG and the 10% PG treatments regardless of concentration of PGA and CG. In addition, the 7.5% and 10% PG treatments had fewer mites than the initial inoculation level of 20 mites, suggesting that mites may not have produced in the jar and that some of the adults from the original inoculation had died. PGA and CG should be included in the coating at 1% since the concentration was thicker and adhered better to the ham surface than the 0.5% treatment. The lowest effective concentration for PG in PGA + CG coatings was 7.5% under laboratory conditions. The XG treatment with 10% PG had fewer mites than the XG treatment without PG ($P < 0.05$) (Table 3.1). In addition, XG with 15% and 20% PG had fewer mites ($P < 0.05$) than the XG + 10% PG treatment. The XG + 15% and 20% PG treatment controlled mites since there were fewer mites than the initial 20 mites that were placed on the ham cubes. The lowest effective concentration of PG that controlled mites was 15% for XG coatings under laboratory conditions. In previous research, incorporation of 20%, 30%, 40% and 50% PG in PGA + CG and XG coatings were effective at controlling mites (Zhao et al., 2016a). Plasma-treated fibers with chitosan/Ag

⁺ coating were toxic to synanthropic mites including *T. putrescentiae* (Rahel et al., 2012). Chitosan alone was not able to achieve a high level of acaricid activity. However, chitosan was used as a delivery method for Ag⁺ (strong toxicity to mites) to inhibit the population growth of *T. putrescentiae* (Rahel et al., 2012). AgNO₃ and Ag₂O are both toxic and not food grade. It is therefore not practical for them to be used on hams. Propylene glycol is generally considered safe and used in the food industry for multiple purposes such as an anticaking agent, an antioxidant, a flavor agent, an emulsifier among other purposes (FDA 21CFR184.1666). PGA + CG serves a similar function to chitosan in that it delivers PG, the active ingredient in the coating. Polysaccharides have been widely studied and used in the food industry as antimicrobial coatings for food packaging including fish and meat products as well as fruits and vegetables (Sánchez-Ortega et al., 2014; Valdés et al., 2017). Alginates used in coatings with sodium lactate (2.4%) and sodium diacetate (0.25%) suppressed the growth of *Listeria monocytogenes* on cold-smoked salmon slices and fillets during 30 d of storage at 4 °C (Neetoo et al., 2010). The PGA (1%) + CG (1%) gum only and XG (1%) treatments demonstrated some inhibitory effects on mite growth as compared to the control ham cubes (Zhao et al., 2016a). However, gums alone were not effective at controlling mite growth, and including propylene glycol as the active ingredient was necessary (Zhao et al., 2016a; Abbar et al., 2016b). Mite orientation experiments conducted by Abbar et al. (2016b) revealed that *T. putrescentiae* avoided staying on or near PG-treated ham pieces and laid very few to no eggs on treated hams, although the mechanism for this inhibitory effect remains to be unknown. Propylene glycol has antimicrobial properties against *Candida albicans*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* A,

Streptococcus mitis and *E. coli* within 20 h (Kinnunen & Koskela, 1991). A study of mite residency on whole hams treated with PG coatings indicated that both 20% PG and 40% PG with PGA + CG treated hams had less than 10 mites ($P < 0.05$) after 6 weeks following the inoculation of 900 mites on each whole ham (Abbar et al., 2016b). This confirms that mites behaviorally avoid these coatings due to an inhibitory effect caused by PG. In summary, these results indicated 7.5% PG or greater for PGA + CG and 15% PG or greater for xanthan gum might be effective at controlling mites on whole hams in future research in commercial processing plants.

3.3.2 Sensory Evaluation on Whole Hams with Food Grade Coatings Applied

3.3.2.1 Difference from control test - trial 1 in 2014

Hams coated with PGA + CG + 20% PG and XG only were not different ($P > 0.05$) from the blind control hams with respect to flavor (Table 3.2). There were slight differences ($P < 0.05$) in the hams treated with PGA + CG + 40% PG, net only, PGA + CG only and XG + 20% PG in comparison to the blind control hams. Even though there was a difference between these treated hams and the control hams, the highest mean rating was 2.7, which indicates a slight to moderate difference. Panelists commented that treated hams were saltier, smokier and had stronger dry-cured flavor than the control hams. In addition, the block (plant) effect was highly variable ($P < 0.05$), since each plant has different processing methods, aging conditions, ham origins and ham size.

Panelists did not detect a difference ($P > 0.05$) in texture between the coated hams and control hams with the exception of the PGA + CG + 40% PG treatment, which was rated moderately different from the control in comparison to the blind control, which was rated slightly different ($P < 0.05$) from the control (Table 3.2).

Hams coated with PGA + CG + 20% PG were not different ($P > 0.05$) from the blind control hams with respect to moistness (Table 3.2). PGA + CG + 40% PG (2.5), net only (2.3) and PGA + CG only (2.2) treated hams were slightly different ($P < 0.05$) from the blind control, which was rated 1.6. Panelists commented that hams from these three treatments were moister. Even though, there were slight differences in the above 3 treatments, the highest rating was 2.5, which is half way between a slight and moderate difference. XG only and XG + 20% PG treated hams did not differ from the control hams with respect to moistness ($P > 0.05$). When developing these coatings, maintaining moisture permeability was crucial since the United States Department of Agriculture requires a dry-cured ham to lose at least 18% of weight from original weight (USDA 9 CFR 319.106). The preliminary weight loss study by Zhao et al. (2016a) on coated whole hams indicated a difference of weight loss within 1% between non-coated and control hams during 2 months of aging.

3.3.2.2 Difference from control test - trial 2 in 2015

Since there were slight sensory differences between some of the treatments and the control in the dipping trial, an additional trial was conducted by spraying coatings on hams in an attempt to lower costs and minimize sensory differences that occurred in Trial 1. Spraying hams led to thinner and more uniform films. Therefore, less coatings were used in the process. There were no differences ($P > 0.05$) in ham flavor, texture, and moistness between treated hams and the control (Table 3.3). All hams including the blind control were rated as slightly different from the labeled control hams. Zhao et al. (2016a) treated 1.3 cm thick ham slices with 100% PG and food grade coatings, and there was no difference among the control and coated ham slices when coated for 2 weeks. The results

of this trial, using treated hams that were then aged six months, confirmed the results from the study by Zhao et al. (2016a). When dipping method was used, there were some differences between treatments and the control. However, in trial 2, spraying was used and treated hams did not differ from the control hams with respect to flavor, texture and moistness. This may be attributed to the spray imparting a thinner coating on the hams with a more consistent coating thickness (Ramos et al., 2012) and controlled delivery of PG, and this may have minimized differences detected by panelists. Thus, the coatings could potentially be applied to hams as a processing aide by spraying to help prevent mite infestations in dry-cured ham processing facilities without negatively impacting sensory properties.

3.3.2.3 Weight loss

No differences ($P > 0.05$) existed in the coated hams in comparison to the control hams with respect to weight loss (Table 3.4). The water vapor permeability was determined for these coatings by Zhao et al. (2016a). A mix of kappa and iota carrageenan was used in the PGA + CG coating. Research by Alves et al. (2006) demonstrated that when kappa- carrageenan concentration was increased in a blend of kappa-carrageenan and pectin, the permeability to gases (O_2 and CO_2) and water vapor also increased. Generally, when plasticizers (e.g. PG) are added to polysaccharide coatings, the permeability to gas and water vapor is increased (Alves et al., 2010; Skurtys et al., 2010), which supports the results on the lack of difference in weight loss in the current study. Zhao et al. (2016a) evaluated moisture loss of hams coated with various food coatings including 100% PG and 2% CG + 50% PG. In that study, hams treated with 2% CG + 50% PG lost 6.4% of weight while the control lost 7.4% of weight after 48

d of storage (Zhao et al., 2016a). The weight loss in this study was measured on 4-month old commercial hams. Therefore, each ham would have already lost 18% of its original weight prior to the receipt of the hams. In the current study, weight loss was not different ($P > 0.05$) among the treated and control hams, but there was variability ($P < 0.05$) among plants (blocking factor) with respect to weight loss, since initial ham weight prior to coating varied in each plant. The hams that were used in both trials had finished curing, and would have already lost greater than 10% of their weight. Therefore, all hams lost greater than 18% of moisture during the combination of curing and aging.

3.3.3 Cost Analysis of Food Grade Coatings

One ham required approximately 500 mL of a given food grade coating solution in our scenario to protect hams from mites. Based on the market price of the ingredients in 2015 (Table 3.5), the price for 1% PGA and 1% CG coatings ranges from approximately \$0.82 to \$2.64 per ham when 10% to 50% PG was used in the coating. The price for 1% xanthan gum coatings varied between approximately \$0.54 and \$2.35 per ham from 10% to 50% PG in the coating. However, these are retail prices and production costs would be much less expensive if bought directly from a company that already produces or sells propylene glycol. According to some processors' price for methyl bromide, it is about \$10 or greater per kilogram, which can be as much as \$3 or more per ham by the time the ham has aged for 18 months to two years (Edwards, personal communication, 2016). In addition, dry-cured ham processors may not have access to methyl bromide once the existing stocks are depleted if there is not an opportunity for the dry-cured ham industry to apply for a critical use exemption for

methyl bromide. Optimization of the coating costs would be necessary to help the dry-cured ham processors reduce production costs and maintain viability.

3.4 Conclusions

Propylene glycol alginate (1%) and carrageenan (1%) based coatings with 7.5% PG and xanthan gum (1%) with 15% PG were effective at controlling mite infestations under laboratory conditions. Dipping hams in coatings led to slight differences in flavor, texture and moistness of dry-cured hams. However, the hams that were sprayed with coatings did not differ with respect to flavor, texture and moistness from the control hams. This implies that dry-cured ham processing facilities could potentially spray these coatings on dry-cured hams to prevent mite infestations in their plants without affecting the sensory quality of the hams. Further research will include incorporating coatings into ham nets to determine their efficacy at controlling mite infestations and their impact on sensory quality.

Table 3.1 Mean number of mites on inoculated ham cubes (20 mites/cube, n = 5) coated with propylene glycol alginate + carrageenan and xanthan gum at different percentages of propylene glycol after 2 weeks incubation at 25 °C and 70% RH.

Gum Treatment	PG	Mean No. of mites	SEM
Control	0%	517 ^a	19
PGA (1%) + CG (1%)	0%	522 ^a	
PGA (1%) + CG (1%)	2.5%	337 ^b	
PGA (1%) + CG (1%)	5%	101 ^c	
PGA (1%) + CG (1%)	7.5%	16 ^d	
PGA (1%) + CG (1%)	10%	4 ^d	
PGA (0.5%) + CG (0.5%)	10%	4 ^d	
Control	0%	270 ^a	20
XG (1%)	10%	80 ^b	
XG (1%)	15%	15 ^c	
XG (1%)	20%	5 ^c	

Means with same letter within the column for each gum (PGA + CG or XG) are not significantly different ($P > 0.05$) using Tukey's Honestly Significant Difference Test at 5% significance level.

Control ham was not coated.

PGA: propylene glycol alginate, CG: carrageenan, PG: propylene glycol, XG: xanthan gum

Table 3.2 Difference-from-control sensory test results by trained panelists (n = 6-10, 12 panels per trial, an average of 100 overall ratings for each treatment for each descriptor) of whole hams (sliced into 1.3 cm thickness) treated by dipping with different food grade coatings after approximately 6 months of aging from 4 plants in 2014

Treatment	Flavor	Texture	Moistness
Blind Control	1.8 ^c	1.7 ^b	1.6 ^d
PGA + CG + 20%PG	2.3 ^{abc}	1.9 ^b	1.8 ^{cd}
PGA + CG + 40%PG	2.5 ^{ab}	2.8 ^a	2.5 ^a
net only	2.4 ^{ab}	2.4 ^{ab}	2.3 ^{ab}
PGA + CG only	2.7 ^a	2.3 ^{ab}	2.2 ^{abc}
XG only	2.1 ^{bc}	2.1 ^{ab}	1.9 ^{bcd}
XG + 20%PG	2.4 ^{ab}	2.2 ^{ab}	1.9 ^{bcd}
SEM	0.039	0.053	0.039

PGA: propylene glycol alginate, CG: carrageenan, PG: propylene glycol, XG: xanthan gum

Means with same letter within each column are not significantly different ($P>0.05$) using Tukey's Honestly Significant Difference Test at 5% significance level.

Scale for sensory evaluation against the labeled control: 1-no difference, 2-slight difference, 3-moderated difference, 4-large difference, 5-very large difference

Table 3.3 Difference-from-control sensory test results by trained panelists (n = 6-10, 12 panels per trial, an average of 100 overall ratings for each treatment for each descriptor) of whole hams (sliced into 1.3 cm thickness) treated by spraying with different food grade coatings at 4 plants after approximately 6 months of aging in 2015.

Treatment	Flavor	Texture	Moistness
Blind Control	1.9	2.1	1.9
XG + 20% PG	2.0	2.3	2.1
PGA + CG + 20% PG	2.2	2.0	1.9
SEM	0.065	0.065	0.042

PGA: propylene glycol alginate, CG: carrageenan, PG: propylene glycol, XG: xanthan gum

Scale for sensory evaluation against the labeled control: 1-no difference, 2-slight difference, 3-moderated difference, 4-large difference, 5-very large difference

Table 3.4 Weight loss of control hams and coated hams after aging approximately 6 months in 4 plants (2 hams/plant, n = 8 each treatment).

Treatment	Weight Loss (%)
Control	16.4
XG + 20% PG	16.7
PGA + CG + 10% PG	18.1
PGA + CG + 20% PG	16.8
PGA + CG only	16.1
SEM	0.40

PGA: propylene glycol alginate, CG: carrageenan, PG: propylene glycol, XG: xanthan gum

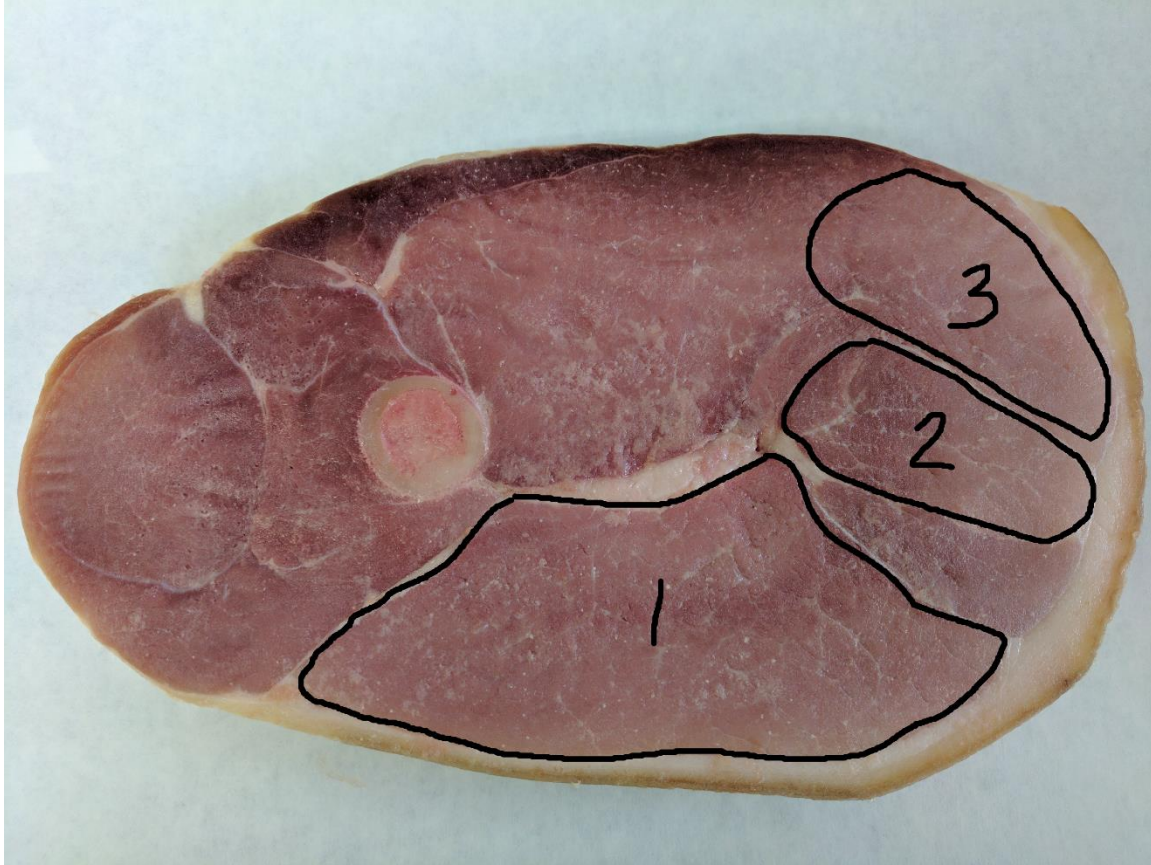
Table 3.5 Cost for coating one ham (500 ml food grade coating solution)

PG percentage	Cost ¹ for 1%PGA + 1%CA coatings	Cost for 1% XG coatings
10%	\$0.82	\$0.53
20%	\$1.28	\$0.99
30%	\$1.73	\$1.45
40%	\$2.19	\$1.90
50%	\$2.64	\$2.35

PG: propylene glycol, PGA: propylene glycol alginate, CG: carrageenan, XG: xanthan gum

¹Cost may vary depending on market cost of ingredient, cost was calculated from the supplier's information.

Figure 3.1 Photograph of a slice from an aged ham typical of those studied, showing the three sampling areas for taste panel evaluations: 1: *M. Biceps femoris*; 2: *M. Semitendinosus* ; 3: part of *M. Semimembranosus*



CHAPTER IV

USE OF NETS TREATED WITH FOOD-GRADE COATINGS ON DRY-CURED HAMS TO CONTROL *TYROPHAGUS PUTRESCENTIAE* INFESTATIONS WITHOUT IMPACTING SENSORY PROPERTIES

4.1 Introduction

Dry-cured hams are produced by rubbing a dry curing mix (salt, nitrite/nitrate) on the hind leg of a hog carcass and allowing the salt to penetrate to the middle of the ham during 6 weeks of storage at 0-4 °C and 2 weeks of cure equalization at approximately 12 °C. After this curing process, hams are placed in nets and aged at 15 °C or warmer for 3 months up to 3 years (Marriot & Ockerman, 2004; Toldrá & Aristoy, 2010). In the United States, more than half of the dry-cured hams that are produced are aged 3 to 6 months. However, many processors keep their hams in the aging house as long as 24 months to produce a ham with the desired flavor profile (Rentfrow et al., 2012). The majority of dry-cured hams are produced in the Southeastern United States, including Tennessee, Georgia, Missouri, North Carolina, Virginia and Kentucky (Rentfrow et al., 2012).

Dry-cured hams may become infested with the ham mite, *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae), during aging. Hams that are aged longer than 6 months have more intense flavor than the shorter aged hams, but also have a higher risk of infestation (Rentfrow et al., 2008). Due to protein and fat composition, water activity, moldy surface, and environment (20-30 °C, 60-80% relative humidity), ham mites can populate in the aging house in approximately two weeks (Rentfrow et al., 2008, 2012; Sánchez-Ramos & Castañera, 2000). Methyl bromide fumigation has been

the standard method for control of ham mite infestations. As of 2008, 22 out of 35 dry-cured ham plants used methyl bromide fumigation to control mite infestations (Rentfrow et al., 2008). However, in 1992, methyl bromide was classified as an ozone depleting substance in the Montreal Protocol (UNEP, 2006). Dry-cured ham processors have since applied for and been issued critical use exemptions to use methyl bromide to fumigate aging facilities through 2016 (EPA, 2015b). However, in 2015, the United States Environmental Protection Agency (EPA) determined that there were sufficient stockpiles of methyl bromide available to U. S. dry-cured pork processors so that a critical use exemption is not currently needed by the industry (EPA, 2015a). Therefore, it is important for the dry-cured ham industry to find effective and economical alternatives due to methyl bromide cost and decreasing availability.

Propylene glycol (100%), a safe and commonly used food additive, is effective at controlling mite growth on ham cubes (Abbar et al., 2016b). In addition, moisture permeable food grade coatings that are formulated with varying percentages of propylene glycol were also effective at controlling mite reproduction on ham cubes (Zhao et al., 2016a) and residency of mites on whole hams (Abbar et al., 2016b). These coatings were dipped or sprayed on whole hams during plant trials needing approximately 500 mL to coat each ham. This technique included an additional processing step and increased labor (Campbell et al., 2017).

Treating different textile fabrics to control pests has been previously evaluated. Rahel et al. (2012) treated polypropylene, non-woven textile with chitosan and metal ions (Cu^{2+} , Ag^+ , Zn^{2+}), and plasma treated fibers with chitosan and Ag^+ controlled the

reproduction of *T. putrescentiae* and other synanthropic mites. Anti-mite modified polypropylene fibers and bedding inserts with these fibers decreased mite populations and inhibited reactions in patients that are allergic to house dust mites (Niekraszewicz et al., 2005). These textiles were treated with heavy metal ions or non-food ingredients and cannot be used on dry-cured hams. Most dry-cured ham processors in the United States hang their hams in nets during aging. Therefore, coatings were infused into ham nets to reduce cost and prevent an additional processing step. The objective of this research was to evaluate the effectiveness of ham nets that were infused with either xanthan gum and propylene glycol or carrageenan, propylene glycol alginate and propylene glycol at controlling mite infestations and evaluate their impact on sensory properties of the hams.

4.2 Materials and Methods

4.2.1 Development of Coated Nets

4.2.1.1 Experiment 1: initial net testing

Polyester nets with stitch density of 170 loops/cm² (Ennio International, Aurora, IL) were coated in a commercial facility in De Pere, Wisconsin. These nets were coated with 1) 100% propylene glycol, 2) propylene glycol alginate + carrageenan + 50% propylene glycol, (PGA + CG + 50% PG), 3) propylene glycol alginate + carrageenan + 20% propylene glycol (PGA + CG + 20% PG), 4) xanthan gum + 20% propylene glycol (XG + 20% PG) for initial testing to evaluate the effectiveness of controlling mite growth on hams. Coatings were made at this facility using methods that were developed by Zhao et al. (2016a). Nets were soaked in the coatings and then fed through a netting machine with an automated double roller system (Midwest Metal Craft & Equipment, Winsor,

MO) (Figure 4.1). This netting machine is commonly used in the netting industry to incorporate liquid smoke into nets that are used in deli ham production, which is referred to as “padding” finish in the textile industry (Hollen & Saddler, 1955; Elsasser, 2005b). Nets were vacuum packaged immediately after “padding”.

4.2.1.2 Experiment 2: screening of nets

A customized netting machine (Midwest Metal Craft & Equipment, Winsor, MO) was made to coat nets in the pilot plant. Since the polyester nets in experiment 1 did not absorb coatings well (Table 4.1), polyester/cotton blend (50/50) and cotton nets from two different commercial netting companies (Plants A and B) were evaluated for their ability to absorb coating (XG + 20% PG and PGA + CG + 20% PG) as well as their effectiveness at controlling mite growth. Polyester/cotton blend nets were referred to as blend nets. Stitch density of blend and cotton nets from plant A were 54 and 69 loops/cm² respectively; stitch density of blend and cotton nets from plant B were 112 and 53 loops/cm² respectively. The greater the stitch density, the finer the net (Elsasser, 2005a), which implies a smaller pore size. Each experiment had a control (without nets), 4 positive controls (untreated nets) and 4 treatments (nets treated with coatings) for a total of 9 treatments for the XG + 20% PG coating and 9 treatments for the PGA + CG + 20% PG coating.

4.2.1.3 Experiment 3: improving net treatments

Nets from plant B were selected for additional testing since they absorbed a greater concentration of coatings and had a greater stitch density than those from plant A. Blend and cotton nets were treated with XG + 10% PG, XG + 20% PG, PGA + CG +

10% PG and PGA + CG + 20% PG to evaluate the effectiveness of these nets at controlling mite population growth when the PG concentration was reduced.

4.2.2 Preparation of Ham Cubes Wrapped with Nets

Dry-cured hams were purchased from a commercial facility for use in mite reproduction assays. Hams were sliced (2.5 cm thickness) and then cut into $2.5 \times 2.5 \times 2.5$ cm cubes. Nets were cut into square pieces to wrap around the cubes (Figure 4.2) and tied with a cotton sewing string to completely cover the cubes. All ham cubes for each experiment were individually stored in 29.5 ml clear plastic containers and then either stored in a Ziploc bag with ice packs and shipped to Kansas State University overnight or stored under refrigerated temperature at Mississippi State University. Each ham cube was inoculated with 20 adult mites on the next day.

4.2.3 Mite Reproduction Assay

Groups of 20 adult *T. putrescentiae* with 10-12 females were inoculated onto each cube from a laboratory colony cultured in the Entomology department at Kansas State University (Abbar et al., 2016b). The cube was placed in a well ventilated glass mason jar (216 ml, 65mm diameter, 55 mm height; Ball Corp., Broomfield, CO) for incubation at 23 ± 2 °C and $70 \pm 5\%$ RH. Mite-inoculated ham cubes were incubated for 14 d to evaluate mite reproduction. Mite populations on the ham cubes were then counted as live adults and mobile immature stages after 2 weeks of incubation.

The mite reproduction assays in experiment 1 were conducted at the Kansas State University (KSU) laboratory. Mite reproduction assays in experiment 2 were then conducted at Mississippi State University (MSU) using the mite colony and methods

from KSU (Abbar et al., 2016b). Assays in experiment 3 were conducted at both MSU and KSU to validate that the mite reproduction assay at the two universities yielded similar results without confounding effects.

In experiment 3, blend and cotton nets from plant B were used to incorporate coatings into the fabric for experiments with XG + PG and PGA + CG + PG. Seventy cubes were prepared and wrapped with nets for each coating with 2 PG concentrations (10% and 20%) and separated into 2 sets of 35 cubes. One set of 35 cubes (7 treatments, $n = 5$) was inoculated with 20 large adult mites (10-12 females) at Mississippi State University and the other set was sent to Kansas State University with the same mite reproduction assay.

4.2.4 Sensory Evaluation-Difference from Control Test

Difference from control tests were used to determine if trained panelists could perceive sensory differences between control ham samples and net-treated samples. Nets treated with XG + 20% PG and PGA + CG + 20% PG were used to hang unaged hams (after curing but prior to aging) as well as control hams in the A.B. McKay building (Enology lab) basement at Mississippi State University. After 4 months of aging at 23-25 °C and 65-75% RH, nets on hams were removed and hams were sliced (1.3 cm thickness) using a band saw (Butcher Boy, Lasar MFG. Company, Inc. Los Angeles, CA, USA). Ham slices were vacuum-packaged and stored at 2 ± 2 °C for 1 to 2 weeks until sensory testing was conducted. Each ham slice was wrapped in aluminum foil and oven-baked at 177 °C to an internal temperature of 71 °C (Marriott & Ockerman, 2004) that was monitored with an infrared thermometer (Horiba IT-330, Horiba Inc, Irvine, CA). Each

ham slice was cut into square pieces with similar sizes (1.3 cm × 1.3 cm) from the same muscle (Figure 3.1). Sensory sampling was mainly from muscle section 1, and two pieces were from section 2 or 3 when needed (Figure 3.1). Upon serving, ham pieces were placed into 29.5 ml clear plastic containers that were coded with 3-digit random numbers. Each panelist was served samples from the same location on the same muscle for each treatment to avoid variation between muscles. Panelists were trained for 2 weeks to evaluate overall differences in flavor, texture and moistness by two faculty members with experience in conducting descriptive panels on dry-cured ham (Pham et al., 2008). Samples were provided in a randomized order to the trained panelists (n= 6-10) with greater than 50 h of experience in tasting dry-cured ham. Water, apple juice, unsalted crackers, napkins, forks, and expectorant cups were provided to the panelists in separate data collection booths for each panel. A blind control was included in each test as a baseline for the random variation between samples. The scale for the difference from control test was: 1 = no difference, 2 = slight difference, 3 = moderate difference, 4 = large difference, 5 = very large difference (Meilgaard et al., 2007).

4.2.5 Statistical Analysis

For the initial net testing in experiment 1, a completely randomized design with five replications was used to evaluate the efficacy of using polyester coated nets to control ham mites. For experiment 2, a 2 × 2 (2 plants × 2 types of nets) factorial arrangement of treatments within a completely randomized design structure with 5 replications of each treatment was used to evaluate differences between commercially available nets and types (blend vs cotton). For experiment 3, a 2 × 3 (2 types of nets × 3

coating levels) factorial arrangement of treatments within a completely randomized design with 5 replications was utilized to determine the effectiveness of different concentrations of propylene glycol and 2 types of nets, with jars as the experimental unit. Proc GLM (SAS 9.4, 2013, SAS Inc, Cary, NC) was used to compare response variables among the different treatments. When differences ($P < 0.05$) occurred among treatments, Tukey's Honestly Significance Difference Test ($P < 0.05$) was used to separate treatment means.

For difference from control sensory tests, a randomized complete block design with three replications was used to evaluate the differences between the control and the netting treated hams, with panelist as the subsample, treatment as the experimental unit and panel as the block. Difference from control rating data was collected through Compusense software (Compusense Cloud, Guelph, CA). Proc GLM (SAS 9.4, 2013, SAS Inc, Cary, NC) was used to compare response variables among the different treatments. When differences ($P < 0.05$) occurred among treatments, Tukey's Honestly Significance Difference Test ($P < 0.05$) was used to separate treatment means.

4.3 Results and Discussion

4.3.1 Development of Coated Nets

4.3.1.1 Experiment 1: initial net testing

The polyester nets that were used in experiment 1 absorbed approximately 32 g of coating per meter of net (Table 4.1, experiment 1) and the absorbencies were not different ($P > 0.05$) among different coating materials. Ham cubes that were placed in polyester nets that were infused with 100% PG and PGA + CG + 20% PG had fewer ($P < 0.05$)

mites than the control (Table 4.2). In addition, the PGA + CG + 50% PG and XG + 20% PG treatments had fewer mites than the 100% PG treatment ($P < 0.05$). This indicates that incorporating these coatings in polyester nets, with PG as the active ingredient slowed down the growth of mite populations on ham cubes. However, since mite numbers were greater than that reported by Zhao et al. (2016a) when only coatings were used, subsequent studies were conducted to determine the efficacy of using blend and cotton nets to control mite infestations.

4.3.1.2 Experiment 2: screening of nets

It was evident that more coating was needed in the nets in order to control the mites. Polyester fibers are hydrophobic and have poor water absorption capacity (Su et al., 2007; Elsasser, 2005c). However, good absorption can be achieved by adding cotton fibers, which are hydrophilic (Su et al., 2007). Therefore, polyester/cotton blend and cotton nets, were evaluated and absorbed between 62 and 272 g of coating per meter of net (Table 4.1, experiment 2).

Since there was no plant \times net interaction ($P > 0.05$), a completely randomized design with one factor was used to analyze data and separate treatment means. Cotton nets had greater ($P > 0.05$) coating absorbance than blend nets within each plant (Table 4.1, Experiment 2). Both blend and cotton nets from plant B absorbed more coating ($P < 0.05$) into the fabric than the nets from plant A, with the exception of the XG + 20% PG treatment (Table 4.1, Experiment 2). For net control treatments, ham cubes wrapped with cotton nets from plant A had fewer mites ($P < 0.05$) than ham cubes wrapped in the cotton net from plant B and the blend net from plant A. In addition, cubes wrapped with

blend nets from plant A and the cotton nets from plant B had a greater number of mites ($P < 0.05$) than the control (Table 4.3). This indicates that uncoated nets did not inhibit mite growth on dry-cured hams, and could even enhance mite reproduction. Cotton and blend nets treated with XG + 20% PG had fewer ($P < 0.05$) mites than the controls. Similar results were obtained for PGA + CG + 20% PG coating. The four net control treatments did not differ ($P > 0.05$) from the control with respect to mite growth on the ham cubes (Table 4.3). However, when these nets were infused with PGA + CG + 20% PG coating, ham cubes had fewer mites ($P < 0.05$) than the control regardless of which net was used. Blend nets from plant B may have less cost than the other nets due to fewer mites ($P < 0.05$) and higher absorbencies ($P < 0.05$). However, since all nets that were infused with coating were effective at reducing mites ($P < 0.05$) to 33 or less in comparison to a few hundred for all control treatments, all four nets would likely be acceptable for use.

Bioactive polymers and fabrics have been used in clothing and bedding to prevent allergic reactions due to house dust mites, such as *Dermatophagoides farina* and *T. putrescentiae*. Tightly woven fabric was recommended for bedding materials because it prevents mite penetration through the material (Mahakittikun et al., 2003; Mahakittikun et al., 2009). Greater than 60% and 100% of the house dust mites died after being exposed to fabrics treated with copper fibers for 1 and 5 d respectively (Borkow & Gabbay, 2004). Fabrics were used as anti-mite activity agents to deliver copper to prevent allergic reactions due to mites in clothing and bedding. Chitosan coating was used to deliver metal ions to control mites and to develop acaricidal materials and/or mite protective food packages. The fibers treated with plasma and chitosan/Ag⁺ were toxic to

T. putrescentiae (Rahel et al., 2012). However, chitosan without Ag⁺ was not effective at controlling mites. Chitosan is widely used in edible films (Rahel et al., 2012; Valdés et al., 2017). However, metal ions such as Cu²⁺ and Ag⁺ are not food grade, and therefore cannot be applied to the dry-cured ham surface. Polysaccharides can be used as edible films on meat products (Sánchez-Ortega et al., 2014; Valdés et al., 2017). Xanthan gum, propylene glycol alginate, and carrageenan form a uniform film (Zhao et al., 2016a) that locks PG in the gel matrix and distributes PG throughout the film. The purpose of the nets in the current study was to deliver coatings that contain PG to inhibit the growth of mites as well as reduce costs to processors that may want to coat whole hams. Incorporating these coatings into net fabrics reached similar inhibitory effects of mite growth in comparison to previous studies by Zhao et al. (2016a) and Campbell et al. (2017). The results from experiment 1 indicated that polyester nets were inefficient to control mite growth on hams, while results from experiment 2 demonstrated that the composition of the nets (cotton or 50% polyester/cotton blend nets) led to increased coating absorbance and subsequent mite control on hams.

4.3.1.3 Experiment 3: improving net treatments

4.3.1.3.1 Xanthan gum

Since there was no interaction ($P > 0.05$) between net type and PG concentration, a completely randomized design was used to analyze data. Blend and cotton net control treatments had fewer mites ($P < 0.05$) than the control treatment without a net (Table 4.4). Use of XG + 10% and 20% PG treatment reduced mite numbers ($P < 0.05$) when compared to the controls ($P < 0.05$). In addition, increasing the PG concentration from

10% to 20% did not increase ($P > 0.05$) the inhibitory effect of the coating. Results obtained at Kansas State University were similar ($P > 0.05$) to the results from Mississippi State University.

4.3.1.3.2 Propylene glycol alginate and carrageenan

Use of cotton nets (cotton net control) reduced mite growth on the ham cubes when compared to the control without a net ($P < 0.05$), but the mite growth for blend net control treatment did not differ ($P > 0.05$) from the cotton net control or the control without a net (Table 4.5). PGA + CG + 10% PG in cotton and blend nets was effective at inhibiting mite growth by keeping the numbers as low as 31 and 62, respectively. The PGA + CG + 20% PG treated cotton and blend nets controlled the mites to counts of approximately 20, with no difference when using cotton or blend nets ($P > 0.05$). In addition, results from KSU and MSU were similar ($P > 0.05$), with the exception of greater mite numbers ($P < 0.05$) on the control treatments at KSU in comparison to MSU.

Results from experiment 3 (Table 4.4 and 4.5) indicates that delivering PG in XG or PGA + CG coatings in cotton or blend nets inhibit mite growth on ham cubes with as little as 10% PG. The minimum concentration of PG that was effective at controlling mite growth was 7.5% in the PGA + CG coating and 15% in the XG coating when the coating was applied to the whole surface of the ham cubes (Campbell et al., 2017). When using ham nets, the fabrics did not cover the whole surface of the ham cubes, so mites can still crawl through the meshes and feed on ham. This might be why some of the numbers in experiment 3 were greater than the initial inoculation level of 20 mites. Further studies

will include increasing PG concentrations in the coatings within the nets and increasing net stitch density to achieve better mite control.

4.3.2 Sensory Difference from Control Tests

There was no difference ($P > 0.05$) detected between the treated hams and the control with respect to flavor, texture and moisture (Table 4.6). However, the average flavor and texture ratings for the PGA + CG treatments were between slightly and moderately different from the control while the XG + 20% PG and blind control were rated as slightly different from the control. These lack of differences are logical since spraying coatings directly on the whole hams (Campbell et al., 2017) and dipping on ham slices (Zhao et al., 2016a) did not impact the sensory properties of the hams. When whole hams were dipped in coatings, there were some differences in flavor, texture and moistness of the treated hams in comparison to control hams (Campbell et al., 2017). When hams were sprayed with these coatings, a thinner and more uniform film was generated, and the treated hams did not exhibit any differences in sensory qualities (Campbell et al., 2017). These referenced results are confirmatory of the results of the current study since less coating was needed to infuse coatings into the nets in comparison to coating the ham surface.

4.3.3 Estimated Cost Analysis of Treated Nets

Based on the retail pricing information provided by the suppliers, treated net costs were approximately \$0.58 for XG + 20% PG and \$0.64 for PGA + CG + 20% PG coatings for each ham (Table 4.7). Spraying coating directly on each ham costs approximately \$0.99 and \$1.28 for XG + 20% PG and PGA + CG + 20% PG coatings,

respectively. These are retail prices and production would be much less expensive for a company that already produces or sells propylene glycol to commercialize the coated nets. In addition, spraying coating directly involves an extra processing step in the production of hams, such as, purchasing ingredients, making the coating and then spraying. Since most ham processors already use ham nets, using polyester/cotton blend or cotton nets infused with these food grade coatings would be a logical inclusion in the production process and in an integrated pest management program.

4.4 Conclusions

Polyester/cotton blend or cotton nets treated with propylene glycol delivered by xanthan gum or propylene glycol alginate + carrageenan were effective at inhibiting and controlling mite growth on ham cubes at concentrations as low as 10%. In addition, use of these nets did not impact the flavor, texture and moistness of the treated whole hams. These treated nets could potentially be used as anti-mite agents to combat mite infestations in aging houses. The next step of this research will include scaling up the technology for use commercial dry-cured ham plants. Additional research will also be conducted to determine how long the nets will remain effective at controlling mites during the aging process.

Table 4.1 Coating absorbencies for each experiment of the netting development by using different types of net fabrics.

Experiment type	Treatment	Absorbance (g/m)
Exp. 1- Polyester nets	100% PG	30
	PGA + CG + 20%	33
	PGA + CG + 50%	30
	XG + 20% PG	36
	<i>SEM</i>	2
Exp. 2 XG + 20% PG Blend and cotton nets from plants A and B	A - cotton	197 ^b
	A - blend	95 ^c
	B - cotton	272 ^a
	B - blend	174 ^b
	<i>SEM</i>	18
Exp. 2 PGA + CG + 20% PG Blend and cotton nets from plants A and B	A - cotton	151 ^{ab}
	A - blend	62 ^c
	B - cotton	197 ^a
	B - blend	138 ^b
	<i>SEM</i>	20
Exp. 3 XG Nets from plant B	10% PG cotton	187 ^{ab}
	10% PG blend	174 ^{ab}
	20% PG cotton	210 ^a
	20% PG blend	167 ^b
	<i>SEM</i>	13
Exp. 3 PGA + CG Nets from plant B	10% PG cotton	200 ^a
	10% PG blend	144 ^b
	20% PG cotton	177 ^{ab}
	20% PG blend	144 ^b
	<i>SEM</i>	17

Means with same letter within each column within each coating for each experiments' treatments are not different ($P > 0.05$) using Tukey's Honestly Significant Difference Test.

PGA: propylene glycol alginate, CG: carrageenan, PG: propylene glycol, XG: xanthan gum

Table 4.2 Mean population growth of *T. putrescentiae* fed on small dry cured ham cubes ($2.5 \times 2.5 \times 2.5$ cm) treated with different concentrations of propylene glycol infused into polyester nets after 2 weeks of incubation at 23 ± 2 °C and $70 \pm 5\%$ RH (n = 5).

Coating type	PG concentration	Mean Mite No.	SEM
Control	NA	405 ^a	38.6
None	100%	300 ^b	
PGA+CG	20%	225 ^{bc}	
PGA+CG	50%	197 ^c	
XG	20%	184 ^c	

Means with same letter within each column are not significantly different ($P > 0.05$) using Tukey's Honest Significant Difference Test at 5% significance level.

PGA: propylene glycol alginate, CG: carrageenan, PG: propylene glycol, XG: xanthan gum

Table 4.3 Mean population growth of *T. putrescentiae* fed on dry-cured ham cubes (2.5 × 2.5 × 2.5 cm) wrapped with cotton and polyester/cotton blend nets from 2 different plants, either A or B, infused with XG + 20% PG and PGA + CG + 20% PG after 2 weeks of incubation at 23 ± 2 °C and 70 ± 5% RH (n = 5).

Coating	Treatment	Mean Mite No.	SEM
XG + 20% PG	Control	223 ^{bc}	36.2
	Ctrl - A cotton	304 ^b	
	Ctrl - A blend	468 ^a	
	Ctrl - B cotton	554 ^a	
	Ctrl - B blend	155 ^c	
	A - cotton	8 ^d	
	A - blend	10 ^d	
	B - cotton	3 ^d	
	B - blend	15 ^d	
PGA + CG + 20% PG	Control	360 ^{abc}	35.0
	Ctrl - A cotton	304 ^{bc}	
	Ctrl - A blend	468 ^{ab}	
	Ctrl - B cotton	554 ^a	
	Ctrl - B blend	155 ^{dc}	
	A - cotton	8 ^d	
	A - blend	33 ^d	
	B - cotton	16 ^d	
	B - blend	29 ^d	

Means with same letter within each column within each coating (XG or PGA + CG) are not different ($P > 0.05$) using Tukey's Significant Difference Test.

PGA: propylene glycol alginate, CG: carrageenan, PG: propylene glycol, XG: xanthan gum

Table 4.4 Mean population growth of *T. putrescentiae* fed on dry-cured ham cubes (2.5 × 2.5 × 2.5 cm) treated with different concentrations of propylene glycol using xanthan gum infused into polyester/cotton blend nets from plant B after 2 weeks at 23 ± 2 °C and 70 ± 5% RH (n = 5).

Xanthan gum Treatment	Mississippi State		Kansas State	
	Mean Mite No.	SEM	Mean Mite No.	SEM
Control	333 ^a	35.3	415 ^a	33.2
Ctrl - cotton	198 ^b		115 ^{bc}	
Ctrl - blend	95 ^{bc}		161 ^b	
10% PG cotton	62 ^c		27 ^c	
10% PG blend	35 ^c		59 ^{bc}	
20% PG cotton	29 ^c		28 ^c	
20% PG blend	20 ^c		21 ^c	

Means with same letter within each column are not different ($P > 0.05$) using Tukey's Honestly Significant Difference Test.

PG: propylene glycol

Table 4.5 Mean population growth of *T. putrescentiae* fed on dry-cured ham cubes ($2.5 \times 2.5 \times 2.5$ cm) treated with different concentrations of propylene glycol using propylene glycol alginate and carrageenan infused into cotton or cotton/polyester blend nets after 2 weeks at 23 ± 2 °C and $70 \pm 5\%$ RH (n = 5).

PGA+CG coating Treatment	Mississippi State		Kansas State	
	Mean Mite No.	SEM	Mean Mite No.	SEM
Control	201 ^a	17.8	480 ^a	34.1
Ctrl - E cotton	137 ^b		214 ^b	
Ctrl - E blend	148 ^{ab}		213 ^b	
10% PG cotton	31 ^c		29 ^c	
10% PG blend	62 ^c		20 ^c	
20% PG cotton	12 ^c		24 ^c	
20% PG blend	24 ^c		34 ^c	

Means with same letter within each column are not different ($P > 0.05$) using Tukey's Honestly Significant Difference Test.

PGA: propylene glycol alginate, CG: carrageenan, PG: propylene glycol

Table 4.6 Difference from control sensory test results of whole hams (sliced into 1.3 cm thickness) treated with blend nets infused with food grade coatings after 4 months aging.

Treatment	Flavor	Texture	Moistness
XG + 20% PG	1.8	1.7	1.9
PGA + CG + 20% PG	2.7	2.2	1.8
Blind Control	2.0	1.7	1.7
SEM	0.12	0.10	0.10

PGA: propylene glycol alginate, CG: carrageenan, PG: propylene glycol, XG: xanthan gum
 Scale for sensory evaluation against labeled control: 1-no difference, 2-slight difference, 3-moderate difference, 4-large difference, 5-very large difference

Table 4.7 Estimated cost using treated nets and in comparison to using coatings on one ham using retail pricing.

Coating type	Cost ¹ for treated nets	Cost ¹ for coating	% of savings
PGA + CG + 20% PG	\$0.64	\$1.28	50.0
XG + 20% PG	\$0.58	\$0.99	41.4

PG: propylene glycol, PGA: propylene glycol, CG: carrageenan, XG: xanthan gum
¹Price may vary depending on market cost of ingredients

Figure 4.1 Netting equipment for infusing food-grade coatings

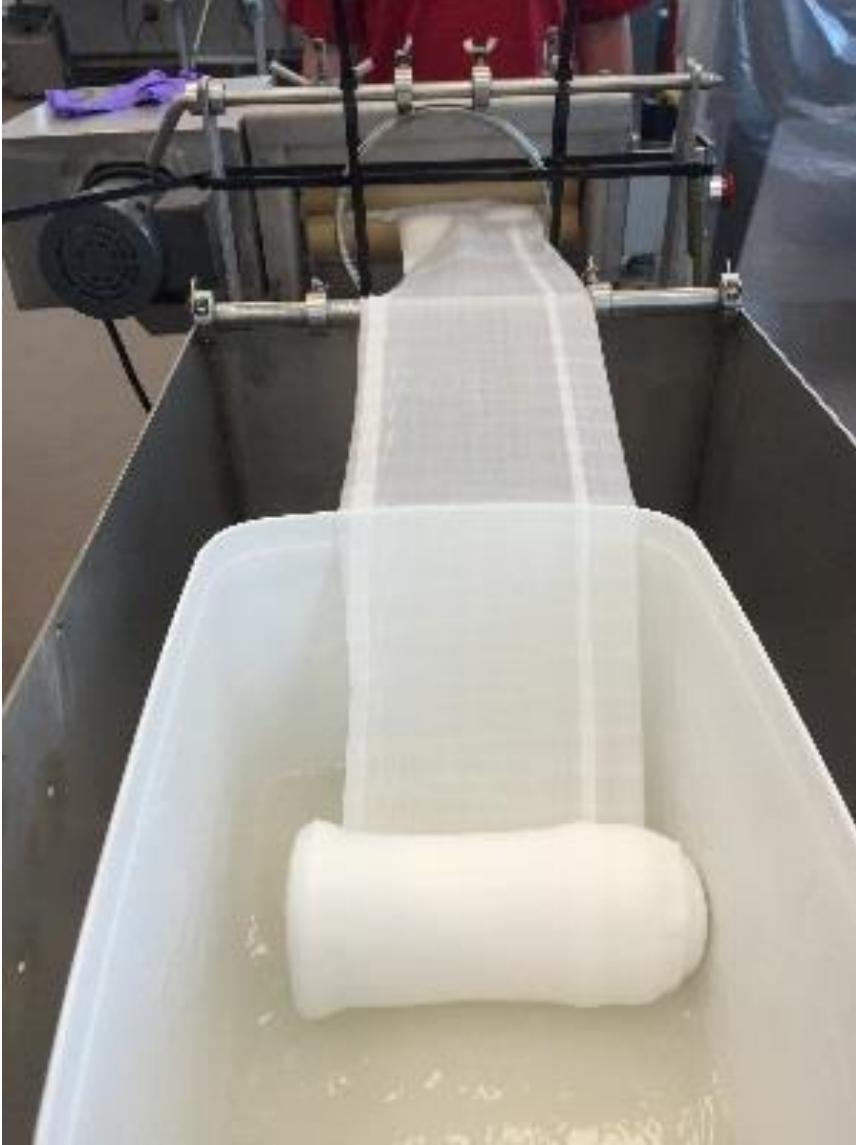
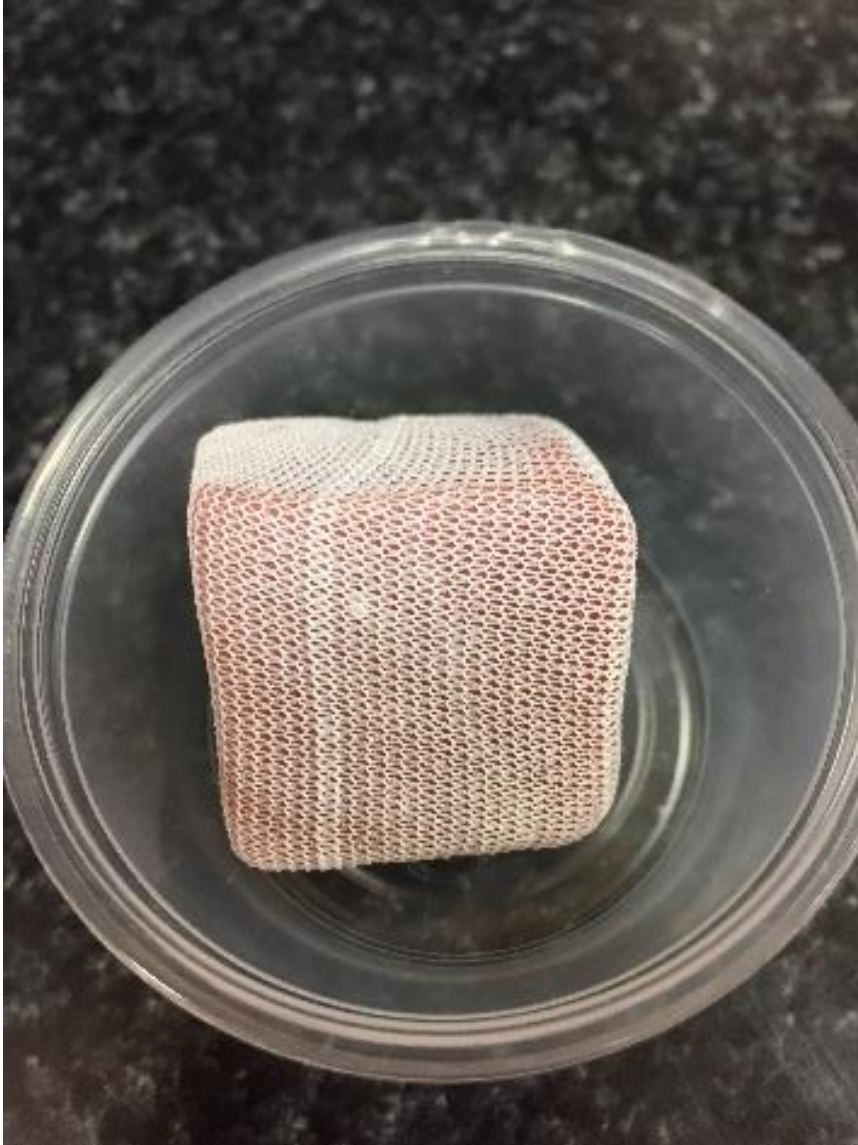


Figure 4.2 Ham cube wrapped with nets treated with food-grade coatings



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