Effects of phosphine fumigation and food-grade coatings on the safety, mite mortality, and sensory quality of dry-cured ham

By

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Dry-cured hams often become infested with ham mites *(Tyrophagus putrescentiae)* during the aging process. Methyl bromide has been used to fumigate dry cured ham processing plants and is the only known fumigant that is effective at controlling ham mite infestations. However, methyl bromide will be phased out of all industries by 2015. This research was designed to 1) determine the efficacy of phosphine fumigation at controlling ham mites and red-legged beetles and its impact on the sensory quality and safety of dry cured hams, and 2) to develop and evaluate the potential of using food-grade film coatings to control mite infestations without affecting the aging process or sensory properties of dry-cured hams. Fumigation trials were conducted in simulated ham aging houses and commercial ham aging houses. Mite postembryonic mortality was 99.8% in the simulated aging houses and >99.9% in commercial aging houses two weeks post fumigation. Sensory tests with trained panelists indicated that there were no detectable differences ($P>0.05$) between phosphine fumigated and control hams. An analytical method was developed to determine phosphine concentration in ham. In addition, residual phosphine concentration was below the legal limit of 0.01 ppm in ham slices

taken from phosphine fumigated hams. Coating trials were conducted on ham cubes and slices. Cubes coated with xanthan gum+20% propylene glycol and carrageenan/propylene glycol alginate+10% propylene glycol were effective at controlling mite infestations under laboratory conditions. Barrier properties (water vapor permeability and oxygen permeability) were measured to estimate the impact of coatings during the aging process. It was evident that carrageenan/propylene glycol alginate were permeable to moisture and therefore could potentially be applied to the hams during the aging process.

DEDICATION

I would like to dedicate this manuscript to my parents, Huijie Zhao and Chunhua Liu, my loving family members in China, and my mentor Dr. Wes Schilling.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Many different types of dry-cured hams are currently produced around the world. American dry-cured ham, also known as country ham, is mostly produced in the southeastern United States including Kentucky, Tennessee, North Carolina, and Virginia (Rentfrow, Chaplin, & Suman, 2012). Dry cured hams are cured, smoked (optional) and aged. Its characteristic flavors and aromas are mainly developed during aging through lipolysis and proteolysis. Aging conditions are variable and the length of the aging process varies from 3 months to 36 months (Toldrá, 2010). *Tyrophagus putrescentiae* (Schrank), also known as the mold or cheese mite, is a widely distributed pest species that infests grains and stored food products such as peanuts, cheese, cotton seed, and dry-cured ham. Dry-cured ham is very susceptible to mite infestations after 4-6 months of aging due to its fat and protein content, water activity, and moldy surface (Rentfrow, Hanson, Schilling, & Mikel, 2008). The environmental conditions where hams are aged also favor mite growth and reproduction. Mold mites have been reported as a problem for dry-cured ham in both Spain (Sánchez-Ramos & Castañera, 2000) and the United States (Rentfrow, Hanson, Schilling, & Mikel, 2008).

Methyl bromide has been used to fumigate commodities and buildings worldwide since the 1930s (Fields & White, 2002) and is the only known fumigant that is effective at controlling ham mite infestations as of 2013 (EPA, 2013). In 1992, methyl bromide

was listed as an ozone depleting substance under the Montreal Protocol, and all developed countries agreed to reduce their amount of methyl bromide use by 2005 (TEAP, 2000). Since 2004, critical uses have been exempted in developed countries on a yearly basis if it was determined that a technically and economically feasible alternative with acceptable environmental and health effects was not available. Thirty-four dry-cured ham processing plants in the United States returned a survey on methyl bromide use, and 22 of these plants reported that their plant fumigated with methyl bromide between one to five times a year, a number that is determined based on the number of times that mites are seen on hams during that year (Rentfrow, Hanson, Schilling, & Mikel, 2008). Since there are no known effective alternatives to methyl bromide for controlling mites in the dry-cured pork industry, a critical use exemption has been granted to the dry cured ham industry since 2005. There was 3,240 kg of methyl bromide approved for dry cured pork products in the United States in 2015 (EPA, 2013). It is important to the economic viability of the dry-cured ham industry to find alternative fumigants and methods to control mite infestations in lieu of using methyl bromide.

Potential alternatives for methyl bromide fumigation include fumigants such as phosphine, physical control methods such as modified atmosphere, pesticides and bioactive compounds such as Storcide II® and limonene from pine essential oils (Abbar, Zhao, Schilling, & Phillips, 2013; Macchioni, Cioni, Flamini, Morelli, Perrucci, Franceschi, & Ceccarini, 2002; Sánchez-Molinero, García-Regueiro, & Arnau, 2010). Prior to 2006, minimal research was published on potential methyl bromide alternatives for dry-cured pork pest control. Since 2006, the effectiveness of phosphine, carbon dioxide, sulfuryl fluoride and ozone have been evaluated under laboratory conditions for

their potential to control ham mites and red-legged beetles (Sekhon, Schilling, Phillips, Aikins, Hasan, Corzo, & Mikel, 2010; Sekhon, Schilling, Phillips, Aikins, Hasan, & Mikel, 2010; Sekhon, Schilling, Phillips, Aikins, Hasan, Nannapaneni, & Mikel, 2010). Among the tested alternatives, phosphine was considered the most promising fumigant to replace methyl bromide. Phosphine fumigation at 1000 ppm in a controlled environment (3.7 liter gas-tight jars at 20°C) achieved 100% mortality of all life stages of ham mite and red-legged beetles with 48 hours exposure. One objective of this study was to evaluate the effectiveness of phosphine fumigation at controlling ham mites under different experimental and real life conditions and to determine the effect of phosphine fumigation on the safety and sensory quality of dry-cured hams.

Edible coatings have been applied for different purposes on a variety of food products including fresh fruits and vegetables, confections and meat products. For meat products, edible films and protective coatings have been used to prevent off-flavor due to oxidation, discoloration, quality loss such as shrinkage, and microbial contamination (Ustunol, 2009). Food grade coatings incorporated with active compounds such as antioxidant and antimicrobials to improve their functionalities have been studied (Arancibia, Giménez, López-Caballero, Gómez-Guillén, & Montero, 2014; Pastor, Sánchez-González, Chiralt, Cháfer, & González-Martínez, 2013; Seol, Lim, Jang, Jo, & Lee, 2009). For instance, alginate and agar bilayer films incorporated with cinnamon essential oil have significantly reduced the microbial growth, including *L. monocytogenes* during chilled storage of peeled shrimp without negatively influencing their organoleptic properties (Arancibia, Giménez, López-Caballero, Gómez-Guillén, & Montero, 2014). It has been stated that coating hams with vegetable oils or hot lard is a common practice in

Spain to control mite infestations in dry-cured ham (García, 2004). However, no research or methodology has been reported to demonstrate how this can be conducted to control mites.

Use of food grade coatings to prevent or stop ham mite infestations could substantially reduce and potentially eliminate the need for chemical fumigations. It is important that the coating materials applied on food products are generally recognized as safe (GRAS) by the Food and Drug Administration and the United States Department of Agriculture. In contrast to most food grade coatings that protect the food product from losing water, a finished ham product needs to lose at least 18% of its original weight during aging (USDA, 1999). In this case, water vapor permeability of the films and coatings needs to be considered when choosing a proper coating for dry-cured ham. Another objective of this research was to evaluate food grade coatings for their efficacy at controlling mite infestations under laboratory conditions and to determine if coatings that are developed would affect the aging process or change the sensory properties of the dry cured ham.

CHAPTER II

LITERATURE REVIEW

2.1 Dry-Cured Ham

The production and consumption of dry-cured pork products started in southern European Mediterranean countries prior to 2000 BC (Toldrá, 2002). People who lived near the Mediterranean Sea discovered that leaving salted meat in dry, cool and windy conditions preserved meat when food was scarce. The practice of dry-cured meat production in Europe was brought to America with the first European settlers. Meat was cured in New England in the 1600s for export to Europe (Kemp, 1982). Many different types of dry-cured hams are currently produced around the world. Some of the most popular dry-cured hams are Iberian and Serrano ham from Spain, Prosciutto ham from Italy, Corsican ham from France, country style ham from the United States, Westphalia ham from Germany, and Jing Hua ham from China (Toldrá, 2010).

American dry-cured ham, also known as country ham, is produced from the hind leg of a pork carcass by rubbing salt and other ingredients that draw out the moisture and break down the myofibrillar protein to achieve its unique cured flavor. A typical American dry cured ham is treated with salt, nitrate and/or nitrite and loses at least 18% of its original weight during processing. The majority of American dry-cured ham facilities are located in the southeastern states and include Virginia, Kentucky, North Carolina, Tennessee, Georgia, and Missouri (Rentfrow, Chaplin, & Suman, 2012).

2.2 Manufacturing of Dry-Cured Ham in the United States

The quality of dry-cured ham is dependent on a number of factors such as breed of hog, feed, age at slaughter, curing procedure, and ham aging procedure. Reddish pink, firm, and non-exudative (RFN) pork is preferred when producing dry-cured ham. Dark, firm and dry (DFD) pork should be treated with caution because of its high pH that facilitates microbial growth. Hogs that have a high degree of marbling are preferred to produce dry cured ham. In the United States, many breeds, such as Duroc and Berkshire, are used to produce country ham (Ockerman, Basu, Crespo, & Sánchez, 2002). Most hogs are fed on corn and soybean meal in the United States. Hogs are usually slaughtered at approximately 6 months of age, which produces hams that are about 7-8kg. However, some producers prefer heavier pigs that yield 9-14kg hams (Ockerman, Basu, Crespo, & Sánchez, 2002).

Dry-cured hams are cured, smoked (optional) and aged. Salt, nitrate and/or nitrite are the major ingredients in the cure mix. Salt inhibits the growth of spoilage microorganisms by reducing the water activity and solubilizing some of the myofibrillar proteins. Salt also provides the salty taste that is characteristic of dry cured ham. Nitrate is reduced to nitrite and further to nitric oxide by nitrate reductase which is a natural enzyme that exists in ham. The typical red/pinkish color of ham is due to the reaction of nitric oxide and myoglobin which forms nitrosomyoglobin (Toldrá, 2002). Other ingredients such as sugar and black pepper are optional for flavor enhancement.

A typical curing mix contains 8 kilograms of salt, 2 to 3 kilograms of sugar, and 90 to 250 grams of sodium nitrate for each 100 kilograms of fresh ham (Ockerman, Basu, Crespo, & Sánchez, 2002). The curing mix is applied on the surface of hams by evenly

spreading or hand rubbing at one time or by three separate applications at certain intervals based on the processors' experience and preference (Ockerman, Basu, Crespo, & Sánchez, 2002). The duration of the ham in the curing mix is typically 6 weeks at 2-4 °C but varies depending on the weight of the fresh ham, the processor's preference and time to meet USDA requirements (USDA FSIS 9 CFR 318.10). Salt penetrates hams at a rate of 2.54 cm of cushion depth per week (Rentfrow, Chaplin, & Suman, 2012). The excess curing mix on the ham surface is removed by washing and soaking hams in water after the curing period. Hams are then transported to a warmer environment (about 13ºC) for approximately 2 weeks for the salt equalization step (Ockerman, Basu, Crespo, & Sánchez, 2002; Rentfrow, Chaplin, & Suman, 2012).

Smoking is optional. Smoking, salting, and drying are three methods that are used to preserve meat. Smoking not only changes the sensory attributes (darker skin color and smoky flavor) but also extends the shelf life. Dry-cured hams are usually cold smoked at below 35ºC for approximately 24 hours. Hot smoke exposes food to a temperature range of 52ºC to 80ºC and thus is not preferred because it would cook the ham or negatively affect shelf life. Hickory, maple, and fruitwoods are popular hardwoods for processors to cold smoke. Many antimicrobial compounds, such as phenols, carboxylic acids and formaldehyde, are produced during the smoking process that prohibits yeast and mold growth on the ham surface (Toldrá, 2010). Phenols also have high antioxidant activities and slow down lipid oxidation that leads to rancid flavor (Toldrá, 2010).

Aging, also known as ripening, is the processing step that develops the unique and characteristic aroma and flavor of country ham. The intensity of the dry-cured aroma and flavor is a result of extensive lipolysis and proteolysis that are proportional to the length

of the aging time. Aging conditions vary based on country of origin (Andres, Ventanas, Ventanas, Cava, & Ruiz, 2005) and individual producers. Aging temperatures usually range between 16 °C and 25 ºC in Europe with relative humidity between 65% and 80% (Toldrá, 2010). In the United States, the aging temperatures are higher, often greater than 28 °C (Rentfrow, Chaplin, & Suman, 2012). Greater than 50 % of U.S. country hams are aged for 3-6 months (Rentfrow, Chaplin, & Suman, 2012), but there are many companies that age hams between 6 and 24 months to develop intense and desirable flavor characteristics. Hams produced in the Mediterranean area such as Iberian hams are aged for a minimum of 18 months (Ockerman, Basu, Crespo, & Sánchez, 2002).

Color, texture and flavor are three major quality characteristics of dry-cured hams. The cured red color is a result of the reaction of myoglobin and nitric oxide which produces nitrosomyoglobin. The higher the concentration of myoglobin, the more intense the cured color will be (Toldrá, 2010). Tenderness is largely due to extent of the key myofibrillar protein breakdown and is directly related to aging time. Structural proteins such as nebulin and titin are greatly proteolyzed during aging (Toldrá, Rico, & Flores, 1993). Other factors, such as the degradation of the connective tissue, the amount of marbling, and the extent of drying also affect the texture of the ham product. Proteolysis and lipolysis are the most important enzymatic reactions that generate compounds responsible for the aroma and taste of dry-cured hams. Proteolysis produces peptides and free amino acids from sarcoplasmic and myofibrillar proteins through enzymatic degradation (Toldra & Etherington, 1988). Free amino acids that are generated from proteolysis directly contribute to the ham flavor. The amount of taste-active amino acids such as aspartic acid, glutamic acid, histidine, valine, arginine, methionine, leucine,

isoleucine, phenylalanine, lysine and tryptophan were found to be highly related to the length of the aging period of Serrano ham (Toldra, 1998). For example, tyrosine and lysine have been related to aged taste and lysine, glutamic acid, leucine, aspartic acid haven been related to the ripened ham taste (Toldrá, 2010). The enzymatic breakdown of muscle lipids contributes to the production of free fatty acids. The free fatty acids are then oxidized to volatile compounds such as aldehydes, pyrazines, ketones, esters, alcohols, and sulfur compounds (Buscailhon, Berdague, Bousset, Cornet, Gandemer, Touraille, & Monin, 1994). These volatile compounds also directly contribute to the aroma and flavor attributes of dry-cured ham.

2.3 Mite Infestation in Dry-Cured Ham

Mites are common pests in stored food products. *Tyrophagus putrescentiae* (Acarina: Acaridae), also known as the mold or cheese mite, is a universal species that infests a great number of grains and stored food products that have high fat and protein content (Gulati & Mathur, 1995). Most mite infestations occur on the surface of food products, however, mites may penetrate inside the product and thus cause more severe economic loss (Zd'árková, 1991). The infested surface sometimes appears to move even when observed by the naked eye due to the massive population of mites, dead mites and cast-off skins may appear around an infested food product (Mueller, Kelley, & VanRyckeghem, 2006). At 60%-80% relative humidity and 20-30 ºC, the mold mite can complete one generation in 8 to 21 days, and the length of the life cycle increases when the temperature drops (Mueller, Kelley, & VanRyckeghem, 2006). Once the temperature drops below 10°C, the mites cannot develop, but they are able to survive at 0°C in an inactive state (Mueller, Kelley, & VanRyckeghem, 2006). The optimal temperature for

females to lay eggs is between 22°C and 26ºC. Females kept on wheat germ or yeast at 20ºC and 85%RH start laying eggs within the first 24h of mating and are able to lay up to 500 eggs throughout their lives (Boczek, 1991). Eggs are more tolerant to adverse conditions such as fumigation since mites are more active and breathe in fumigant gas, whereas the egg stage has a low level of respiration and is therefore less susceptible to fumigants (Phillips, Thoms, DeMark, & Walse, 2012).

Dry-cured ham is very susceptible to mite infestations due to its high fat and protein content and its moldy surface. The environmental conditions where hams are processed and stored also favor mite growth. Ham mites have been considered the most serious pest problem for dry-cured ham producers both in Spain (Sánchez-Ramos & Castañera, 2000) and the United States (Rentfrow, Hanson, Schilling, & Mikel, 2008).

Producers who age hams for approximately three months are less likely to have mite infestations than those that are aged for longer than 5 months. The intensity of dry-cured ham flavor is proportional to the aging time. Due to the market demand for long-aged hams that have more intense flavor, some producers age hams for one year or longer. A survey conducted on mite prevalence in 34 dry-cured ham plants in the United States (North Carolina (14), Virginia (4), Kentucky (8), Georgia (1), Tennessee (3), and Missouri (4)) indicated that mite infestations occur more often in hams that are aged for 5 to 6 months or longer (Rentfrow, Hanson, Schilling, & Mikel, 2008). Out of the 19 processors that age their hams longer than 6 months, 15 reported the presence of mites on hams; out of the 19 plants that age their hams less than 6 months, 10 reported the presence of mites on hams (Rentfrow, Hanson, Schilling, & Mikel, 2008). Even though proper sanitation was helpful for pest control, the producers that experience mite

infestations reported that methyl bromide fumigation was the only effective way to control ham mites (Rentfrow, Hanson, Schilling, & Mikel, 2008).

2.4 Means to Control Mite Infestations

2.4.1 Methyl Bromide

Methyl bromide is an odorless, colorless gas that has been used to control pests in commodities and buildings worldwide since the 1930s for its broad spectrum application and rapid action (Fields & White, 2002). It not only diffuses homogeneously within the enclosed space at ambient temperature but is also able to penetrate into pore spaces. Methyl bromide kills mites, insects, nematodes, and microflora rapidly without contaminating the products and is nonflammable and noncorrosive (Bond, 1984). Methyl bromide's mechanism of action is to damage the membrane of nerve cells in pests (Fields & White, 2002). In the United States, methyl bromide has been primarily used for soil fumigation prior to planting fruits and vegetables such as tomatoes and strawberries, for post-harvest storage, for facility fumigation, and for quarantine purposes (Osteen, 2003).

The Montreal Protocol is an international treaty signed by the United States and 182 other countries that is designated to protect the ozone layer through phasing out the use of substances that contribute to the depletion of the ozone layer that protects the earth and its inhabitants from ultraviolet radiation (Osteen, 2003). Antarctic ozone levels were reduced by 50% from the early 1970s to the mid-1990s and the reactive forms of bromide were considered to cause half of the loss of the ozone layer over the Antarctic (Rosskopf, Chellemi, Kokalis-Burelle, & Church, 2005). The protocol has gone through seven revisions; methyl bromide was listed as a class one ozone depleting substance in 1992 during the third revision. In the United States, approximately 27,000 metric tons (MT) of

methyl bromide was used each year prior to 1992. Soil fumigation contributed to 75% of the total usage, followed by 11% usage for commodity treatments, 6% for structural fumigation, with the remaining percentage for feedstock in industrial chemical production (Johnson, Walse, & Gerik, 2012). The phaseout was scheduled to reduce methyl bromide use by 25% by 1999, 50% by 2001, 70% by 2003 and 100% by 2005 (with the exception of critical use exemptions) (Osteen, 2003). Only yearly requests for critical use exemptions, quarantine and pre-shipment applications, and emergency uses are exempt from the phaseout (Johnson, Walse, & Gerik, 2012). In 2014, pre-plant strawberries (415MT), post-harvest commodities (0.74 MT), post-harvest structures (22.8 MT), and dry-cured ham (3.7MT) were nominated for critical use exemptions. For pre-plant strawberries, black root rot or crown rot disease, yellow/purple nutsedge infestation, and/or nematode infestation are the three major conditions that require the critical use (EPA, 2012c). Post-harvest structures of rice millers and pet food manufacturers were exempted in 2014 due to beetle, weevil, moth, or cockroach infestation and the presence of sensitive electronic equipment that are susceptible to corrosion (EPA, 2012b). Post-harvest commodities such as walnuts, figs, dried plums, and dates were exempted in 2014 since rapid fumigation is required for these commodities to meet critical marketing seasons (EPA, 2012a). Dry-cured pork products have received a critical use exemption due to red-legged beetle, ham skipper, dermestid beetle and/or ham mite infestations.

The survey of 34 dry-cured ham processing plants mentioned above indicates that 22 of the 34 plants surveyed used methyl bromide fumigation one to five times a year based on the number of infestations that were experienced in their facility (Rentfrow, Hanson, Schilling, & Mikel, 2008). When the first methyl bromide consumption

reduction began, the price increased from $2.50/lb in 1999 to $4.50/lb in 2001 (Osteen, 2003) and has continued to increase over the years. In 2014, methyl bromide prices reached greater than $20/lb. This increase in price has made it difficult for small processors to afford methyl bromide. Methyl bromide fumigation is important to the economic viability of the dry-cured ham industry, which justifies the need for research to determine if alternative methods can be developed to prevent and control mite infestations. Since there are no registered effective alternatives for methyl bromide in the dry-cured pork industry at this time, there was 3,240 kg of methyl bromide approved for dry cured pork products in the United States in 2015 (EPA, 2013).

2.4.2 Alternatives for Methyl Bromide

The phase out process of methyl bromide has created a need for researchers to evaluate potential alternatives to this fumigant. For pre-plant soil applications, several alternative fumigants have been registered in the United States such as chloropicrin (Pic), 1,3-dichloropropene (1,3-D), metam sodium, iodomethane, and dimethyl disulfide, which are currently used in combination (Johnson, Walse, & Gerik, 2012). Research in Florida and California suggested that these alternative combinations can be as effective and economical as methyl bromide for many crops, and there has been increased acceptance by growers to adopt these combinations (Ajwa & Trout, 2004; Gilreath, Santos, & Motis, 2008; Gilreath, Santos, Motis, Noling, & Mirusso, 2005; Johnson, Walse, & Gerik, 2012; Sydorovych, Safley, Welker, Ferguson, Monks, Jennings, & Louws, 2008). For post-harvest and structural applications of methyl bromide, sulfuryl fluoride and phosphine are registered fumigants that are considered alternatives to methyl bromide for the fumigation of commodities and processing facilities. Fumigation studies indicate that

insect eggs are more tolerant to sulfuryl fluoride than methyl bromide (UNEP, 2011; Walse, Leesch, & Tebbets, 2009). Other possible alternatives such as ozone, ammonia, ethane dinitrile, carbonyl sulfide, and methyl iodide have demonstrated effectiveness under some conditions; more studies are needed to verify their efficacy to obtain approval for FDA registration and to receive industry acceptance (Johnson, Walse, & Gerik, 2012).

Other non-fumigant alternatives to control pests in commodities and structures include integrated pest management (IPM), cold treatments, heat treatments, contact insecticides, controlled/modified atmosphere, and inert dusts. The concept of IPM is defined by the FAO Panel of Experts as "a pest management system that, in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible and maintains the pest population at levels below those causing economic injury" (Kogan, 1998). The main focus for an IPM program to succeed is prevention, which includes using physical barriers to prevent insects from entering, frequent cleaning and sanitation, and controlling the environmental temperature and humidity. Another key focus of IPM is to monitor pest populations and to make sure that the preventive actions are effective through inspections. Most ham producers in the United States fumigate when mite infestations are visually observed. However, a bait trap was recently developed to detect and monitor mite populations in dry-cured ham processing plants, which may assist future decisions on when to fumigate (Amoah, Schilling, & Phillips, 2013). Rentfrow et al (2008) suggested a list of good manufacturing practices to country ham processors for preventing pest infestations, such as 1) a gravel dead zone (no grass or shrubs that can harbor pests) at least 2 feet immediately around the aging house; 2) keep the space

outside the plant free of garbage and debris and keep the outdoor trash away from the aging house; 3) keep areas inside the ham plant clean and sanitized; 4) regularly clean the walls and floors of the aging house so that the moisture and fat that has accumulated on those surfaces will not harbor mites; 5) personnel who discover mite infestation should change clothes and shower to avoid cross contamination; 6) clean and sanitize the aging house and racks thoroughly at the end of each aging period prior to hanging a new batch of hams. Cold treatments can be applied as part of an IPM program for stored products. The population of most pests in durable products starts to decline slowly at 10ºC with ceased reproduction. At -15°C, most pest species in durable commodities will be controlled after a few days (UNEP, 2001). Cold treatments can be combined with other treatments. For example, in combination with reduced oxygen, insects were expelled out of the center of fruits at 2.8% oxygen which reinforced the effect of the cold treatment (Donahaye, Rindner, & Dias, 1992; Donahaye, Navarro, & Rindner, 1991). It's hard for contact insecticides to replace methyl bromide entirely due to their poor penetration of commodities, insect resistance, possible toxicity to humans and animals, and residues in food. Modified atmospheres control pest infestations by reducing oxygen content. The advantages are minimal safety issues and lack of residues in food. The disadvantages are slow action and the need for airtight facilities. Hermetic storage, nitrogen treatments, and carbon dioxide treatments are three ways to control storage pests. Heat treatments have fast action without undesirable residues; however, commodities that will be damaged by heat cannot be treated.

Research has been carried out to verify the efficacy of potential alternatives to control storage mites. There were no visible mites on hams which were processed in a

reduced oxygen (O_2<4.5%) atmosphere until after 275 days when mites were detected on control hams (Sánchez-Molinero, García-Regueiro, & Arnau, 2010). Reduced oxygen increased the proteolysis index and decreased the cholesterol oxide concentration. When reduced oxygen (O_2<4.5%) was combined with RH 50%, the growth of bacteria and fungi on ham surfaces were slower than the control (resting in air with RH 70%-80%). Insect growth regulators are not applicable options since they have limited effects on acarine pests (Collins, 2006). Many synthetic pesticides and organophosphate acaricides are recommended to control storage mites; however, they are restricted in the food industry due to residue concerns (Sánchez-Ramos & Castañera, 2003). Inert dusts have the potential to control mite infestations. As pests walk over a treated surface, they pick up the dust and the dust functions by physically removing or absorbing their cuticular waxes, which lead to loss of water from the body and desiccation (Ebeling, 1971). Storage mites breathe through cutaneous respiration and are weakly sclerotized and are thus vulnerable to desiccation (Collins, 2006). Inert dusts have traditionally been used as a storage grain protectant (Golob & Webley, 1980). However, the effective dose amount that is required is very high (Collins, 2006). For instance, 1g/kg of dust was needed to control 95% of mold mites in stored cereals (Collins, Armitage, Cook, Buckland, & Bell, 2001). The effect of inert dust against mites on dry-cured hams has not yet been evaluated. Mite control agents that contain various bioactive compounds that are derived from plant extracts may be potential alternatives as well. Many plant extracts, such as azadirachtin from the neem tree, fennel seed oil, cinnamon and pine essential oil (Kim, Kim, & Ahn, 2004; Lee, Sung, & Lee, 2006; Macchioni, Cioni, Flamini, Morelli, Perrucci, Franceschi, & Ceccarini, 2002; Sánchez-Ramos & Castañera, 2000) have been

reported to have the potential to control storage mites under laboratory conditions. However, none of these compounds have been tested on dry cured hams. Kim et al., (2004) tested the acaricidal activity of cinnamaldehyde and its 11 derivatives against adults of *T. putrescentiae* with a direct contact method and found that the lethal dose (LD50: concentration to kill half the population) of cinnamylacetate (0.89 ug/cm^2), cinnamaldehyde (1.12 μg/cm^2) and five other congeners were much lower than the commonly used benzyl benzoate (10.03 μg/cm^2) and diethyltoluamide (DEET) (13.39 μg/cm^2). Lee et al (2006) tested the acaricidal activities of fennel seed oil derivatives against *T. putrescentiae* adults also using a direct contact method. Compared with benzyl benzoate (11.24 μg/cm^2), the LD50 of fennel seed oil (+)-carvone was 4.62 μg/cm^2. Macchioni et al (2002) studied the acaricidal activity of the main components of pine essential oils against *T. putrescentiae* and found that all the essential oils showed good efficacy, especially 1,8-cineole and limonene which had 100% acaricidal activity through aerial diffusion. A piece of Parma ham harboring more than 20 mites was placed in a 6-cm petri dish which 6uL of 1,8-cineole which resulted in 100% acaricidal activity. Sánchez-Ramos and Castañera (2000) tested 14 monoterpenes and 7 of them were effective against mobile stages of *T. putrescentiae* by vapor action. However, none of them were effective against eggs. They also found that males and larvae had significantly higher mortality than females when exposed to similar concentrations of monoterpenes. So far, none of these bioactive compounds have been tested directly on dry-cured meat products. When applying these bioactive compounds on dry-cured hams, one major concern would be how they would affect the sensory quality of dry-cured hams since they all have distinct aromas.

2.4.3 Potential Alternative Fumigants for Ham Mites

Minimal research was published on potential methyl bromide alternatives for dry-cured pork pest control prior to 2006. Since 2006, the effectiveness of phosphine, carbon dioxide, sulfuryl fluoride and ozone have been evaluated under laboratory conditions for their potential to control ham mites and red-legged beetles (Sekhon, Schilling, Phillips, Aikins, Hasan, Corzo, & Mikel, 2010; Sekhon, Schilling, Phillips, Aikins, Hasan, & Mikel, 2010; Sekhon, Schilling, Phillips, Aikins, Hasan, Nannapaneni, & Mikel, 2010). Although sulfuryl fluoride controlled 100% of all life stages of the red-legged ham beetle at below 1,500 g-hrs/m^3 (label rate), it was not effective against the egg stage of ham mites even when applied at 3 times the label rate (Phillips, Hasan, Aikens, & Schilling, 2008). However, more recently it has been reported that sulfuryl fluoride was more effective against mite eggs as the temperature increased from 20 °C to 40 °C (Phillips & Schilling, 2013). Ozone applied at 175 ppm for 48 hrs had 100% lethality against all life stages of ham beetles and mites under laboratory conditions. However, in real world practice, ozone was not considered effective due to its lack of ability to penetrate ham surfaces, where ham mites and ham beetles could potentially exist during pest infestations. However, two processors that are members of the National Country Ham Association currently use ozone and are evaluating its efficacy in their plants. To achieve 100% mortality for all life stages of ham mite and red-legged beetles, the concentration of carbon dioxide was required to be kept at 60% or above for 144 hour. This is not economically feasible since a ham plant could not close for more than 6 days and remain economically viable (Hasan, Phillips, Aikins, & Schilling, 2010). Phosphine applied at 1000 ppm in a controlled environment (3.7 liter gas tight jars at 20 °C) achieved 100%

mortality of all life stages of ham mite and red-legged beetles with 48 hours exposure (Sekhon, Schilling, Phillips, Hasan, Aikens, & Mikel, 2009). Similar results were observed on the efficacy of phosphine and sulfuryl fluoride fumigation at controlling mites on dog food under laboratory conditions. The mite mortality was less than 50 % at 7,071 ppm sulfuryl fluoride. However, fumigation with 381 ppm phosphine for 72 hours led to approximately 100% mite mortality (Mueller, Kelley, & VanRyckeghem, 2006).

Phosphine is the most widely used fumigant alternative for methyl bromide (Fields and White, 2002) due to its easy application, effectiveness, low cost and lack of residue (Zuryn, Kuang, & Ebert, 2008). The density of phosphine is 1.17 times that of air and it is a gas at above -88 °C, which makes it disperse well during fumigation (Nath, Bhattacharya, Tuck, Schlipalius, & Ebert, 2011). Phosphine is highly toxic to aerobically respiring organisms, but is not toxic to either anaerobic or metabolically dormant organisms (Nath, Bhattacharya, Tuck, Schlipalius, & Ebert, 2011). Phosphine works as a strong reducing agent in the mitochondrial electron transport chain of aerobic respiring organisms. By disrupting normal oxygen metabolism, it induces the production of highly toxic 'oxyradicals' and other intermediates (Bolter and Chefurka, 1990). These toxic compounds cause the malfunction of vital enzymes that are essential for metabolism (Zuryn, Kuang, & Ebert, 2008). Ham mites can survive in low oxygen environments. Anaerobic respiration will be triggered since reduced aerobic respiration cannot satisfy the energy demand. Accumulated anaerobic end products cause stress to mites (Mitcham, Martin, & Zhou, 2006). The drawbacks that prevent phosphine from being an ideal fumigant include: slow activity which translates to a longer fumigation period, the need for a highly sealed enclosure, increased pest resistance, flammability above 1.8% in

volume, and corrosion to certain metals such as copper (Benhalima, Chaudhry, Mills, & Price, 2004; Price and Mills, 1988; Zettler, Halliday, & Arthur, 1989).

2.5 Phosphine Residue Determination

2.5.1 Phosphine Residue Extraction

The legal concentration of phosphine residue in processed food products is 0.01 ppm or less (40 CFR 180.225). Phosphine is highly volatile and disperses easily. Some researchers suggested that residues from phosphine fumigation could be eliminated rapidly by ordinary aeration after a certain period of time (Al-omar & Al-bassomy, 1984) while other researchers reported that residue may persist in the food product (Norman & Leonard, 2000). The majority of research on this topic has been focused on phosphine fumigation residue in stored grains since phosphine is the predominant fumigation chemical to control pests in stored grains (Nath, Bhattacharya, Tuck, Schlipalius, & Ebert, 2011).

To determine residue concentration, phosphine must first be extracted from the food matrix prior to the analysis. In early work, phosphine residue was determined by titration or colorimetric techniques that are much less sensitive than gas chromatographic analysis. Phosphine can be extracted from the commodity matrix by 1) purge-and-trap; 2) solvent extraction; and 3) microwave irradiation (Daft, 1991; Heikes & Hopper, 1986; Heuser & Scudamore, 1969; Ren & Desmarchelier, 1998). In a purge-and-trap system, an inert gas such as helium or nitrogen is purged through an aqueous sample which leads to the liberation of insoluble volatile compounds from the food matrix. The volatiles are retained in the trap, and then desorbed for further analysis. However, this method is not adequate for highly volatile compounds such as phosphine because it is unable to trap one

hundred percent of the fumigant (Ren & Desmarchelier, 1998). Solvent extraction depends on the solubility of compounds in a food matrix. It is also a time consuming process with results that may be affected by solvent interference (Ren & Padovan, 2010). Microwave extraction has shown good recovery and precision for phosphine extraction. However, water vapor generated from microwave heating can significantly interfere with gas chromatography results and damage very expensive columns (Ren & Mahon, 2007).

Solid-phase micro extraction (SPME) was developed in 1990 (Kataoka, Lord, & Pawliszyn, 2000) and has since become a versatile extraction method for many applications (Peñalver, Pocurull, Borrull, & Marcé, 2001). This technique is sensitive, effective, simple and does not need a solvent. The SPME device looks like a modified syringe. Inside the needle part there is a fused-silica fiber that is coated with a thin film that is the stationary phase and functions like a sponge to extract and concentrate analytes. This method not only saves extraction time, but also lowers detection limits (Kataoka, Lord, & Pawliszyn, 2000). For instance, when analyzing methy lisothiocyante residue after fumigation, the residue from the wheat sample can be analyzed at below 0.1 ppm successfully using an SPME fiber while the limit of detection for the purge and trap method was 10 ppm (Ren, Lee, Mahon, Xin, Head, & Reid, 2008; Ren & Padovan, 2010).

Selecting the appropriate fiber coating and adequate extraction time will affect the performance of the SPME analysis. The principle of SPME is based on the “like dissolves like” theory. Coating fibers are designed with different thicknesses and materials to efficiently extract compounds with different polarities. A polydimethylsiloxane (PDMS) fiber is suitable for the extraction of non-polar compounds while polyacrylate (PA) is used for polar analytes. Mixed polarity coating fibers, such as

PDMS- Divinylbenzene (DVB) and carboxen (CAR: porous activated carbon support)-PDMS promote retention capacity by mutual potentiation of adsorption and distribution to the stationary phase. Also, agitation, adding salt to the sample, and optimizing extraction temperature and pH enhance extraction (Kataoka, Lord, & Pawliszyn, 2000).

2.5.2 Residue Detection with Gas Chromatography

The flame photometric detector (FPD) is one of the most commonly used selective detectors in gas chromatography. The FPD has a reducing flame that produces chemiluminesent species. These species emit characteristic light that can be optically filtered for the desired wavelength (Wilson & Klee, 1997). For example, if phosphorous is the desired analyte, the interference from substances that do not contain phosphorous will be minimized by the filter. FPD is frequently used to analyze specific compounds in complex samples. Although the wavelength filter can be set to select for many elements, FPD is most often utilized for sulfur and phosphorous detection (Wilson & Klee, 1997).

Pulsed flame photometric detector (PFPD) uses a pulsed flame instead of a continuous flame, which has several significant improvements when compared to the FPD. When using a PFPD, the flame is ignited, propagated and self-terminated 2-4 times per second. Each specific element has its own emission profile. Take phosphorous compounds as an example; hydrocarbons complete emission early while phosphorous emissions start at a later time after combustion. As a result, a timed “gate delay” will selectively allow for emissions due to phosphorous only. This timed “gate delay” greatly improves the sensitivity of the analysis (Jing & Amirav, 1998). The combination of SPME with GC–PFPD can enhance the efficacy to detect phosphine residue at relatively low concentrations.

2.6 Food-Grade Coatings

2.6.1 Introduction

As early as the 12th century, wax coatings were developed in China for use on fresh citrus fruits so that the emperor in the north could enjoy citrus grown in southern China (Hardenburg, 1967). A similar process was developed in Europe to preserve fruits in wax or fat, which was called "larding" (Labuza & Contreras-Medellin, 1981). The layers formed from both practices protected fruits from water loss and interfered with gas exchange. Even with the development of many advanced technologies for food protection and preservation, coating with edible film is still one of the most cost-effective ways to maintain the safety and quality of many types of food (Pavlath & Orts, 2009).

Edible coatings have been applied for many different purposes on a large variety of food products such as fresh fruits and vegetables, confections and meat products. For example, sucrose esters are used to extend the shelf life of melons; cellulose derivatives are used to reduce fat uptake during the frying of fish and potatoes; shellac is coated on M&Ms to provide glaze and extend shelf life; medicine is often coated to mask bitterness and prevent crumbing (Pavlath & Orts, 2009). Most research on the coating of meat products has been focused on preventing water loss and reducing rancidity (Baldwin, 2007). Generally, lipids are applied to reduce water permeability; protein layers provide mechanical stability; polysaccharides are employed to regulate gas transmission (Pavlath & Orts, 2009).

Edible films can be made by 1) spraying/brushing the solution with film ingredients or dipping the product into a solution; and 2) making stand-alone film and then covering it on the surface of the food. A plasticizer, such as glycerol, is usually

added to the coating solution to keep the film from being brittle (Pavlath & Orts, 2009). It is important that the coating materials applied on food products are generally recognized as safe (GRAS) by the Food and Drug Administration and the United States Department of Agriculture.

Edible films and protective coatings have been used to prevent oxidative off-flavors, discoloration, quality loss such as shrinkage, and microbial contamination in meat products (Ustunol, 2009). In the sixteenth century, cut meats were coated with fats to reduce moisture loss and shrinkage in England (Ustunol, 2009). Bee wax and carnauba were used to coat frozen meat to lengthen the shelf life. Besides lipid coatings, polysaccharide film coatings such as carrageenans, pectins and alginates have been used to improve meat quality. Extended shelf life was reported for meat products that were coated with algin, dextrose and a calcium solution (Ustunol, 2009). Methylcellulose has been used to produce glazed sauces to minimize moisture loss of poultry and seafood during cooking (Baker, Baldwin, & Nisperos-Carriedo, 1994). Protein film coatings such as gelatin have been used in poultry products to prevent moisture loss and microbial growth and reduce oil absorption during frying (Ustunol, 2009). Films with good barrier properties have been developed with gelatin solutions incorporated with polyhydric alcohols such as glycol and propylene glycol (Whitman & Rosenthal, 1971). The most successful commercial applications of edible film coatings in the meat industry are reconstituted collagen films and sausage casings (Ustunol, 2009). Collagen films are also used as wraps in netted roasts and hams (Gennadios, Hanna, & Kurth, 1997).

2.6.2 Importance of Coating Permeability

Use of food grade coatings to prevent or stop ham mite infestations could substantially reduce and potentially eliminate the need for chemical fumigations. To be qualified as a coating for dry-cured ham, the compound must 1) be food grade; 2) be able to attach to the ham surface; 3) be able to cover the ham surface evenly; 4) be stable during the aging process; 5) be permeable to oxygen and water vapor; 6) be able to suffocate and/or repel mites and insects when applied properly; 7) not adversely affect ham flavor; 8) be easily removed after the aging process.

There are three steps in a diffusion process when food is covered with a coating. Take water migration as an example. First, a water molecule comes in contact with the surface of the film layer; second, water diffuses through the layer; finally, when the water molecule arrives at the other side of the film, it desorbs (Pavlath & Orts, 2009). Ideally, Fick's law is used to determine the total amount of a given material (Q) passing through a film:

$$Q = PA\Delta pt/d \tag{2.1}$$

Q increases in direct proportion to film surface area (A) and time (t), and decreases as the film thickness (d) increases. P is the permeability coefficient of the film. Δp is the pressure difference between the two sides of the film that affect Q linearly (Pavlath & Orts, 2009). This equation is only applied under ideal conditions. In real world applications, many factors, such as varying temperature and an uneven film layer, will affect the total amount of permeate.

In contrast to most food grade films that protect the food product from losing water, a finished ham product needs to lose at least 18% of its original weight during

aging (USDA, 1999). In this case, water vapor permeability of the films and coatings needs to be considered when choosing a proper coating for dry-cured ham. Ideally, the permeability coefficient (P) will be a constant that is determined by film characteristics and structure (Pavlath & Orts, 2009). Generally speaking, highly polar substances with a strong affinity for hydrogen bonding are poor barriers to moisture and have low gas permeability. Different rates of evaporation of the coating solution may produce films with different characteristics. For instance, permeability may be affected when polymer chains are prematurely immobilized by accelerated drying before reaching their optimal structure (Greener, 1992).

Oxygen permeability is also important since oxygen concentration affects 1) the intensity of nitrosylmyoglobin and thus the color of dry-cured ham; 2) lipid oxidation and thus the flavor attributes of dry-cured ham; 3) proteolysis and thus the texture and the flavor profile of dry-cured ham. The reduction of oxygen may also have positive effects on dry-cured ham quality since reduced oxygen will have an adverse effect on mold development as well as mite growth (Sánchez-Molinero, García-Regueiro, & Arnau, 2010). Although the intensity of musty odor is reduced, hams processed in a reduced oxygen environment received more negative feedback in sensory panels due to production of a white film, metallic and pigsty flavor, and a less intense aged flavor (Sánchez-Molinero, García-Regueiro, & Arnau, 2010).

2.6.3 Polysaccharide-Based Food-Grade Coatings

The purpose of developing a food grade coating for dry-cured ham is to prevent mite infestation with minimum influence on moisture loss, proteolysis, and lipid oxidation during aging. An ideal film coating should have a relatively high water vapor

and oxygen permeability when forming a film coating on the ham surface. Compared with lipid-based films, protein-based films and polysaccharide films have higher water permeability due to their hydrophilic characteristics (Miller & Krochta, 1997). Protein-based films have lower oxygen permeabilities than non-ionic polysaccharide films (Talens, Fabra, & Chiralt, 2010). Polysaccharide-based films are more prone to disintegrate after water absorption (Cuq, Aymard, Cuq, & Guibert, 1995), and thus generally have weaker barrier properties than protein-based coatings (Talens, Fabra, & Chiralt, 2010). Polysaccharide films show good gas barrier properties if no plasticizers are added. The gas permeabilities increase significantly as the content of plasticizer increases (Alves, Costa, & Coelhoso, 2010). Therefore, a film coating made of polysaccharides is more likely to be an ideal coating for dry-cured ham. The barrier properties of hydrophilic polysaccharides are strongly related to their water content and thus the humidity of the surrounding environment (Bertuzzi, Castro Vidaurre, Armada, & Gottifredi, 2007).

Polysaccharide based coatings have the potential to be used in many food applications such as to extend the shelf-life of seafood, meat, fruits and vegetables through preventing oxidation, rancidity, dehydration and surface browning (Talens, Fabra, & Chiralt, 2010). Polysaccharides include starches and their derivatives, cellulose and its derivatives, gums, pectin, chitosan, and seaweed extracts. Starch is derived from plant tubers and seed endosperm and is one of the most abundant natural polysaccharides. It has two types of molecules; amylose imparts film-forming properties and amylopectin imparts thickening and viscosity (Romero-Bastida, Bello-Pérez, García, Martino, Solorza-Feria, & Zaritzky, 2005). Starch films are transparent or translucent, tasteless and

colorless (Skurtys, Acevedo, Pedreschi, Enrione, Osorio, & Aguilera, 2010). Unmodified starches have limited application in the food industry as compared to modified starches, which is due to retrogradation of unmodified starch during cooling and/or storage. The water vapor permeability of films made of high methyl amylose corn starch significantly increased as the environmental temperature increased between 5 °C to 40 °C (Bertuzzi, Castro Vidaurre, Armada, & Gottifredi, 2007). Cellulose is a structural component of the primary cell wall of green plants (Talens, Fabra, & Chiralt, 2010). Methyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, and carboxymethylcellulose are cellulose derivatives that are used to create edible coatings (Skurtys, Acevedo, Pedreschi, Enrione, Osorio, & Aguilera, 2010). These films are transparent, odorless, tasteless, colorless, flexible, water-soluble, and are moderate barriers to oxygen.

Many different types of seaweed extracts are used in the food industry. Carrageenan, agar and alginate can all be used as edible coatings. Alginates can form a biopolymer film coating due to its unique and well-studied colloidal properties which include thickening, film formation, gelation producing, and stabilizing properties in both films and emulsions (Skurtys, Acevedo, Pedreschi, Enrione, Osorio, & Aguilera, 2010; Talens, Fabra, & Chiralt, 2010). Edible films made from alginate form strong films and have poor water resistance due to their hydrophilic nature (Kester & Fennema, 1986). Alginates are suitable to load additives and antibacterial compounds in edible films (Skurtys, Acevedo, Pedreschi, Enrione, Osorio, & Aguilera, 2010). Carrageenans are galactose polymers that are extracted from red seaweed. Carrageenans are widely used in food and pharmaceutical industries as gelling and stabilizing agents. The three main carrageenan types are kappa, iota and lambda which differ in the number and position of

sulphate groups. Unlike kappa and iota, lambda is unable to form gels and is used as a thickener. Agar forms strong gels and is best known as a culture medium and is therefore not widely used in foods.

There are three groups of gums: exudate gums, seed gums and microbial fermentation gums. Gum arabic is an exudate gum that is the least viscous of hydrocolloid gums (Skurtys, Acevedo, Pedreschi, Enrione, Osorio, & Aguilera, 2010) and is used in confections and as a foam stabilizer (Talens, Fabra, & Chiralt, 2010). Guar gum and locust bean gum are examples of seed gums that are extensively used in the food industry including bakery, meats, beverages, confections and dairy products. They can be used as thickeners, viscosity modifiers, suspending agents and stabilizers in different food products. Xanthan gum is produced from *Xanthomonas campestris* fermentation. It is widely used for thickening, stabilizing, and suspending ingredients in salad dressings, sauces, syrups, icings and frostings, and baked products (Talens, Fabra, & Chiralt, 2010). Two polysaccharides can be combined together to improve functionality and reduce cost. For example, chitin and guar gum were combined together due to the antimicrobial activity of chitin and excellent physical properties and inexpensive price of guar gum (Rao, Kanatt, Chawla, & Sharma, 2010). Locust bean gum and k-carrageenan have been used together to enhance tensile strength and increase water vapor permeability (Martins, Cerqueira, Bourbon, Pinheiro, Souza, & Vicente, 2012). However, further studies are needed to employ these combinations in commercial food systems.

The water vapor permeability (WVP) of methylcellulose (MC) films plasticized by polyethylene glycol increased from 0.232 to 1.160×10^{-10} g/m s Pa with increased concentration of polyethylene glycol and ethanol (Nazan, Turhan, & Sahbaz, 2004).

Olivas and Barbosa-Canovas studied the WVP of alginate films with different plasticizers under different relative humidity differentials. Results suggested the lowest WVP occurred at 0 % RH with less than 76 % fructose and the highest WVP occurred with less than 100 % propylene glycol at 0 % RH (Olivas & Barbosa-Cánovas, 2008). Rao et al (2010) reported that films that were made with different chitosan and guar gum ratios had similar water vapor transmission rates. They also reported that films made from pure chitosan had the highest oxygen transmission rate which was 1846 $ml/m^2/day$ (Rao, Kanatt, Chawla, & Sharma, 2010). It had been suggested that plasticizers such as glycerol could increase the WVP and gas permeability of starch film by interacting with starch chains to reduce packing between chains (Mali, Grossmann, García, Martino, & Zaritzky, 2004). Both the WVP and OP of yam starch films plasticized with glycerol increased as glycerol concentration increased. At 2% (w/w) glycerol, the OP of 0.07 mm thickness yam starch films was 3.85×10^{-10} $cm^3\ m^{-1}\ s^{-1}\ Pa^{-1}$ (Mali, Grossmann, García, Martino, & Zaritzky, 2004). The WVP of films made of 1%- 2% chitosan and 1% methylcellulose in different proportions ranged between $7.55\text{-}9.03\times10^{-11}$ $g\ s^{-1}\ mPa^{-1}$ (García, Pinotti, Martino, & Zaritzky, 2004). The barrier properties of polysaccharide films that have been modified and produced with different methods have been studied with respect to plasticizer type and concentration, drying conditions, and environmental conditions (Aydinli & Tutas, 2000; Jost, Kobsik, Schmid, & Noller, 2014; Rhim, 2004; Silva, Bierhalz, & Kieckbusch, 2009; Srinivasa, Ramesh, Kumar, & Tharanathan, 2004).

2.6.4 Conventional and Potential Food-Grade Coatings to Control Mite Infestations on Dry-Cured Hams

Several years ago, cured meat was rubbed with a paste of lard on the surface prior to storage to prevent flies and bacteria (Smith, 1923). It has been stated in a book that coating hams with vegetable oils or hot lard is a common practice in Spain to control mite infestations in dry-cured ham (García, 2004). However, no research or methodology has been reported to demonstrate how this can be conducted to control mites.

The properties of food-grade coatings incorporated with active compounds such as antioxidants and antimicrobials to improve their functionalities have been studied. The antioxidant activity of films made from methylcellulose and chitosan was directly proportional to the amount of resveratrol that was incorporated into the films (Pastor, Sánchez-González, Chiralt, Cháfer, & González-Martínez, 2013). The physical properties of the films changed after adding resveratrol, such that there were slightly decreased barrier properties, increased opaqueness, decreased glossiness, loss of stretch ability and decreased resistance to fracture (Pastor, Sánchez-González, Chiralt, Cháfer, & González-Martínez, 2013). Film coatings made from k-carrageenan incorporated with ovotransferrin (a protein of avian egg's antimicrobial defense system) and EDTA were applied on fresh chicken breast and have shown inhibition against *E. coli* and reduced total aerobic bacteria during storage (Seol, Lim, Jang, Jo, & Lee, 2009). Both alginate and agar bilayer films incorporated with cinnamon essential oil have significantly reduced the microbial growth, including *L. monocytogenes* during chilled storage of peeled shrimp without negatively influencing their organoleptic properties (Arancibia, Giménez, López-Caballero, Gómez-Guillén, & Montero, 2014). Pullulan films with sweet basil extract have shown good antifungal activity against *Rhizopus arrhizus* on

apple surfaces as well as protection from weight loss, change in color and soluble solids during storage (Synowiec, Gniewosz, Kraśniewska, Przybył, Bączek, & Węglarz, 2014). Many other researchers have studied the effects of incorporating active antimicrobials and antioxidants in food grade film coatings (Espitia, Avena-Bustillos, Du, Teófilo, Soares, & McHugh, 2014; Ferreira, Nunes, Castro, Ferreira, & Coimbra, 2014; Jouki, Yazdi, Mortazavi, & Koocheki, 2014; Norajit, Kim, & Ryu, 2010; Noronha, de Carvalho, Lino, & Barreto, 2014; Teixeira, Marques, Pires, Ramos, Batista, Saraiva, & Nunes, 2014). Development of food-grade coatings with compounds added to control mite reproduction could be a non-fumigation alternative to control mite infestations that can potentially occur on dry-cured ham.

A list of animal and vegetable oils (including soybean oil, canola oil, corn oil, olive oil, mineral oil and lard), sorbic salts (sodium/potassium/calcium sorbate, propionic salts (sodium/potassium/calcium propionate), iodide salts (sodium/potassium/calcium iodide), citrate salts (sodium/potassium/calcium citrate), short chain alcohols (1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 1,2-propanediol, 1,3-propanediol, 1-propanol, 2-propanol), organic acids (including maleic acid, citric acid, 3,3-thiodipropionic acid) and butylated phenol preservatives (BHT and BHA) have been tested as dippings on dry-cured ham cubes (Abbar, Schilling, & Phillips, 2012). Among all of the tested substances and concentrations, 100% lard, 50% propylene glycol (1,2-propanediol) and 10% butylated hydroxytoluene (BHT) were effective at controlling mite reproduction under laboratory conditions (Abbar, Schilling, & Phillips, 2012). Propylene glycol is an odorless, colorless liquid and is often used as a solvent for many organic compounds. As a registered bacteriostat and fungistat, the target pests are: odor-causing bacteria, mites,

fleas, animal pathogenic bacteria, red lice, *Shigella* bacteria, *Listeria* spp., mold, *Aspergillus niger* fungus, and many others (EPA, 2006). It is used widely in personal care products (Huntsman, 2009) and non-food indoor uses like air treatment, medical premises, air conditioning, and pet treatment (EPA, 2006). Propylene glycol has also been used as a humectant in soft-moist dog foods and has been reported to be effective at controlling mite infestations (Aldrich, 2014; Phillips, 2011). Propylene glycol and ethylene glycol were investigated as a trapping and killing agents for arthropod pitfalls. Propylene glycol was able to sequester 53 species out of 85 that were studied, 8 of which were not caught in other traps (Weeks & McIntyre, 1997). The Food and Drug Administration and the Flavor and Extract Manufacturers Association consider propylene glycol to be generally recognized as safe (GRAS) and approved as a food additive for all food categories up to 2% (FAO, 1974). Propylene glycol is metabolized into lactic acid and pyruvic acid, which are produced during normal glycolysis in the body (Thomas, 2008). Propylene glycol could be potentially applied with polysaccharides as a processing aid to form a consistent film coating on the surface of dry-cured hams during the aging process to control mite growth.

Based on the Code of Federal Regulations 21CFR101.100, food processing aids are defined as: "(a) Substances that are added to a food during the processing of such food but are removed in some manner from the food before it is packaged in its finished form; (b) Substances that are added to a food during processing, are converted into constituents normally present in the food, and do not significantly increase the amount of the constituents naturally found in the food; (c) Substances that are added to a food for their technical or functional effect in the processing but are present in the finished food at insignificant levels and do not have any technical or functional effect in that food."

Propylene glycol (also called propane-1,2-diol) is in the list of all compounds used as processing aids in the Codex Alimentarius-Inventory of Processing Aids (CAC/MISC 3). Coatings made of polysaccharides and propylene glycol, according to the definition, if removed completely before packaging hams for distribution, could be considered as a processing aid during the aging process.

Adding propylene glycol as an active ingredient in polysaccharide edible films may be a promising way to control mite infestations during the ham aging process. When compared to lard, an edible coating composed of polysaccharides and propylene glycol is more permeable to oxygen and water vapor, which allows product aging and is easier to remove from the product after aging. It is important to demonstrate the effectiveness of this new processing aid for the control of mite infestations; equally important tasks are to evaluate if the new process is economically viable for processers to adopt and to obtain industry acceptance.

2.7 Sensory Evaluation

It's very important to evaluate the effects of fumigation and other treatments for pest control on the sensory profile of dry-cured hams. Sekhon et al (2010) reported that phosphine fumigation at 1000 ppm phosphine for 48 h in 10.3 L pickle jars, consumers could not discriminate between the control and fumigated hams (Sekhon, Schilling, Phillips, Aikins, Hasan, Corzo, & Mikel, 2010). When compared to the traditional drying process, drying of hams in a reduced oxygen atmosphere (O_2<4.5%) prevented mite growth; however, it had a negative effect on the sensory attributes and was thus considered inappropriate for use in the production of traditional dry-cured hams (Sánchez-Molinero, García-Regueiro, & Arnau, 2010). For ready-to-eat dry-cured hams,

irradiation doses below 5.0 kGy increased the overall sensory acceptability among trained panelists (Jin, Kim, Chung, Jo, Jeong, Hur, Jung, Joo, &Yang, 2012).

Difference tests can be divided to two groups: overall difference tests and attribute difference tests. Overall differences tests, such as the triangle test, duo-trio test, and difference from control test, are used to determine if a sensory difference exists between samples (Meilgaard, Civille, & Carr, 2007). Attribute difference tests are used to evaluate a single attribute difference between samples. Difference from control tests can be used to determine: 1) if a difference exists between treated samples and control; and 2) the magnitude of the difference (Meilgaard, Civille, & Carr, 2007). Each panelist is provided with a control sample and one or more test samples. Panelists are asked to evaluate the size of difference between each sample and the control. Mean differences from the control are compared with the degree of difference between a blind control and the control. By using a blind control, the numerical effects of simply asking the difference question when no actual difference exists will be estimated. The panelists should be familiar with the meaning of the evaluation scale, and the test format. In addition, panelists could be trained or untrained, but should not be a mix of the two (Meilgaard, Civille, & Carr, 2007).

One limitation of difference tests is that the characteristics of the differences are not clear or defined. Descriptive analysis is thus used to define the differences detected from the difference tests. Descriptive analysis is a sensory evaluation technique that includes discrimination and description of both qualitative and quantitative sensory aspects of the product by trained panelists (Meilgaard, Civille, & Carr, 2007). The qualitative factors that are used to define the sensory profile of a sample include:

appearance characteristics (color, surface texture, size, shape, and interaction among pieces or particles), aroma (olfactory sensations such as fruity and floral, and nasal feeling such as pungent), flavor (olfactory sensations such as vanilla and rancid; basic taste such as salty and sweet; and oral feeling such as astringent and metallic), oral texture (such as hardness, gritty, flaky, greasy and moist), skinfeel (such as ease to spread and gritty and greasy) (Meilgaard, Civille, & Carr, 2007). The Flavor Profile Method, Texture Profile Method, Quantitative Descriptive Analysis (QDA®) Method and SpectrumTM Descriptive Analysis Method are commonly used descriptive methods. In the QDA method, panelists are selected based on their sensitivity to discriminate differences in the sensory properties of different samples. Product and ingredient references are used to develop a sensory language and panelists need to be familiar with the language after training. Sensory attributes such as appearance, aroma, flavor and texture are evaluated on 15 cm line scales in the QDA method. In the Spectrum analysis, panelists use absolute intensity scales to evaluate intensities of characteristics for both training and testing (Meilgaard, Civille, & Carr, 2007)

For dry-cured hams that are treated with methyl bromide alternatives, difference from control tests can be carried out to determine if trained panelists can distinguish between treated and control hams. If differences are detected, descriptive analysis can be utilized to describe these differences. Subsequent consumer panels could then be conducted to determine if consumers object to the changes caused by an alternative treatment to methyl bromide.

CHAPTER III

PHOSPHINE FUMIGATION AND QUANTITATION IN DRY-CURED HAM IN COMMERCIAL APPLICATIONS

3.1 Introduction

American dry-cured ham, also known as country ham, is mostly produced in the southeastern United States including Kentucky, Tennessee, North Carolina, and Virginia (Rentfrow, Chaplin, & Suman, 2012). Dry cured hams are cured, smoked (optional) and aged. Its characteristic flavors and aromas are mainly developed during aging through lipolysis and proteolysis. Many processors in the United States age their ham between 3 and 6 months, but some processors age hams between 6 months and 24 months to obtain a premium product with characteristic aged ham flavor. *Tyrophagus putrescentiae* (Schrank), also known as the mold or cheese mite, is a universal species that infests grains and stored food products such as peanuts, cheese, cotton seed, and dry-cured ham. At 60%-80% relative humidity and 20-30 ºC, the mold mite can complete one generation in 10 to 24 days. The optimal temperature for females to lay eggs is between 22ºC and 26ºC. Females kept on wheat germ or yeast at 20ºC and 85% RH start laying eggs within the first 24 h of mating and are able to lay up to 500 eggs throughout their life (Boczek, 1991). Dry-cured ham is very susceptible to mite infestations after 4-6 months of aging due to its fat and protein content, water activity, and moldy surface (Rentfrow, Hanson, Mikel & Schilling, 2008). The environmental conditions where hams are aged also favor

mite growth and reproduction. Mold mites have been reported as a problem for dry-cured ham in both Spain (Sánchez-Ramos & Castañera, 2000) and the United States (Rentfrow, Hanson, Schilling, & Mikel, 2008).

Methyl bromide has been used to fumigate commodities and buildings worldwide since the 1930s (Fields & White, 2002) and is the only known fumigant that is effective at controlling ham mite infestations as of 2013 (EPA, 2013). In 1992, methyl bromide was listed as an ozone depleting substance under the Montreal Protocol, and all developed countries agreed to reduce the amount of methyl bromide that they used by 2005 (TEAP, 2000). After 2004, critical uses were exempted in developed countries on a yearly basis if a technically and economically feasible alternative with acceptable environmental and health effects was not available. Since there are no known effective alternatives for methyl bromide for controlling mites in the dry-cured pork industry, a critical use exemption has been granted to the dry cured ham industry ever since 2005 with 3,730 kg approved for dry cured pork products in the United States in 2014 (EPA, 2013). Thirty-four cured ham processing plants in the United States returned a survey on methyl bromide use, and 22 of these plants reported that their plant fumigates with methyl bromide between one to five times a year, a number that is determined based on the number of times that mites are seen on hams during that year (Rentfrow, Hanson, Schilling, & Mikel, 2008). Since methyl bromide is the only known treatment that is effective at controlling mites, methyl bromide fumigation is important to the economic viability of the dry-cured ham industry. Therefore, there is a need for research on potential alternative fumigants and methods to control mite infestations in lieu of using methyl bromide.

The effectiveness of phosphine, carbon dioxide, sulfuryl fluoride and ozone have been evaluated under laboratory conditions for their potential to control ham mites and

red-legged beetles (Sekhon, Schilling, Phillips, Aikins, Hasan, Corzo, & Mikel, 2010; Sekhon, Schilling, Phillips, Aikins, Hasan, & Mikel, 2010; Sekhon, Schilling, Phillips, Aikins, Hasan, Nannapaneni, & Mikel, 2010). Phosphine fumigation at 1000 ppm in a controlled environment (3.7 liter gas-tight jars at 20°C) achieved 100% mortality of all life stages of ham mite and red-legged beetles with 48 hours exposure. Phosphine is registered for more than 50 raw commodities, processed foods, and nonfood items in the United States (Phillips, Thoms, DeMark, & Walse, 2012). The most common phosphine delivery method is to use metallic phosphide salts which react with water vapor in the air to generate phosphine gas. Magnesium phosphide and aluminum phosphide are used to generate phosphine gas through reacting with water in the following reactions:

$$AlP + 3H_2O \rightarrow Al(OH)_3 + PH_3\uparrow \quad (3.1)$$

$$Mg_3P_2 + 6H_2O \rightarrow 3Mg(OH)_2 + 2PH_3\uparrow \quad (3.2)$$

The minimum ideal ambient condition for the reactions is 27ºC and 70% RH or higher (Phillips, Thoms, DeMark, & Walse, 2012).

The maximum legal amount of phosphine residue allowed in processed food products is 0.01 ppm (40 CFR 180.225). To determine the amount of residue, phosphine needs to be extracted from the food matrix prior to the analysis. Solid-phase micro extraction (SPME) is a simple, sensitive and effective extraction method with a wide range of applications (Peñalver, Pocurull, Borrull, & Marcé, 2001) that is used in combination with gas chromatography and is widely used in environmental analysis, food analysis and pesticide residue analysis (Kataoka, Lord, & Pawliszyn, 2000; Pragst, 2007; Ren, Padovan, & Desmarchelier, 2012; Vas & Vekey, 2004). Polydimethylsiloxane (PDMS) is the non-polar phase and polyacrylate (PA) is polar absorbent phase.

Adsorbent types work by physically trapping the analytes in the internal pores. Analytes are retained in the pores by hydrogen bonding or Van der Waals interactions. The adsorbent type fibers contain either Carboxen (CAR) and/or divinylbenzene (DVB) which are suspended in a liquid phase to enhance bonding of the adsorbent to the fiber. CAR can only be suspended in PDMS. DVB can be suspended in either PDMS or Carbowax (CW) (Shirey & Mindrup, 1999). PDMS/DVB, CAR/PDMS, and DVB/CAR/PDMS are all bipolar and were selected for extraction based on the polarity, molecular weight and concentration of the analytes. The objectives of this study were 1) to evaluate the effectiveness of phosphine fumigation at controlling ham mites under different experimental and real life conditions; 2) to develop an analytical method to quantify phosphine concentration in ham; 3) to determine the effect of phosphine fumigation on the residual phosphine concentration and sensory quality of dry-cured ham.

3.2 Materials and Methods

3.2.1 Fiber Selection and HS-SPME Extraction Time

Phosphine gas (0.5ppm) was purchased from MESA International Technologies, INC (Santa Ana, CA). PH_3 gas was transferred to 1 liter gastight Tedlar bag (Sigma-Aldrich, INC., Bellefonte, PA) through a stainless steel regulator (MESA International Technologies, INC., Santa Ana, CA) and a 0.5 cm diameter rubber hose. The gas flow of the regulator was 1 liter per minute, thus 500 ml of phosphine was transferred to each Tedlar bag after 30 sec. Four SPME fiber coatings were used to extract phosphine from the Tedlar bags: 1) 65 μm Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) (Supelco, Bellefonte, PA. Cat. 57310-U), 2) 75 μm Carboxen/Polydimethylsiloxane (CAR/PDMS)

(Supelco, Bellefonte, PA Cat. 57318), 3) 85 µm Carboxen/Polydimethylsiloxane (CAR/PDMS) (Supelco, Bellefonte, PA Cat. 57334-U), and, 4) 2 cm 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Bellefonte, PA Cat. 57348-U). All fibers were conditioned according to the manufacturer's instruction sheet daily prior to testing.

Six different lengths of extraction periods were tested to optimize extraction time. SPME fibers were exposed to 500 ml of 0.5 ppm phosphine gas in Tedlar bags for 5, 10, 15, 20, 25, and 30 min. The fiber was then placed in the injector of a GC-pFPD and desorbed for 5 min. Triplicate samples for each extraction time were tested. The GC-pFPD analysis was performed using a Varian CP-3800 (Varian Inc., Walnut Creek, CA) gas chromatograph that was equipped with a pulsed flame photometric detector (pFPD) with a phosphorous filter. A PoraBOND Q column (25m*0.53 mm i.d., Agilent Technologies, Inc., Santa Clara, CA) was used to separate the volatile components. The injector and detector temperature were set at 200 °C, and nitrogen was used as the carrier gas at a rate of 5.0 ml/min. The column temperature was set at 125 °C and held for 5 min for each run. The response in the GC-pFPD was shown as peak area as either uVolts or mVolts.

3.2.2 Ham Preparation and Fumigation

3.2.2.1 Laboratory Fumigation Studies

Three commercial aged hams were cut into 2.54 cm thick slices. One slice from each ham was fumigated in 3.8 liter gas-tight glass jars targeted at 1000 ppm phosphine gas. A compressed phosphine cylinder (1% phosphine in nitrogen, Matheson TriGas, Basking Ridge, NJ) was used to generate phosphine in the jars. Another slice from each

ham was packaged in zip lock bags as controls for future sensory and residue tests. The concentration of phosphine inside the jars after 24 and 48 hrs of fumigation were determined by generating a standard curve using 200 ppm phosphine. Three injections of 200ppm in GC-PFD were carried out with each of the following volumes: 5ul, 10ul, 15ul, 20ul, and 25ul to make the standard curve. From each jar, 15ul of gas sample was analyzed three times. After 48h fumigation, jars were brought outdoors for aeration. After one min aeration, 6 ham pieces (approximately 10 grams per piece) from each ham were sealed in 40ml gas-tight glass vials. The remainder of the hams were cut into 10g pieces and aerated for 2 days and/or 4 days for phosphine residue detection.

3.2.2.2 Fumigation in Simulated Ham Aging House

Three fumigation trials were conducted in 30 m^3 shipping containers that were used to simulate dry-cured ham aging houses. During each trial, three shipping containers were used to perform a control, a low fumigation treatment and a high fumigation treatment. Phosphine gas was produced in the shipping containers using magnesium phosphide cells that reached target fumigation doses 8 to 12 hrs after the fumigation was started. In the first trial, the low fumigation concentration was targeted at 1000 ppm for 48 h and the high fumigation concentration was targeted at 2800 ppm for 48h. In the second trial, the low fumigation concentration was targeted at 2000 ppm for 48 h, and the high fumigation concentration was targeted at 2500ppm for 48h. In the third trial, the fumigation time was extended to 72 h with both concentrations in the low and high treatment targeted as the same as those in the second trial to examine the effect of extended exposure time.

Phosphine concentration was measured in the shipping containers using both Draeger- Tubes® (Phosphine, Draeger Safety Inc., Pittsburgh, PA), and a calibrated UV-VIS detector (ATI PortaSens II Gas Detector; Analytical Technology Inc., Collegeville, PA). Twenty ham mite bioassays and 10 red-legged beetle bioassays were evenly distributed in the center and corners in each shipping container. Ten dry cured hams were hung in each shipping container. The lean portions of 5 hams were inoculated with approximately 1000 mites on each ham; another 5 hams were uninoculated and later used for sensory testing. The ambient temperature was recorded by Hobo® data loggers. After fumigation, the shipping containers were left open for aeration. The hams were left in the containers for 24 h after the container had been aerated to a concentration of phosphine below 0.3 ppm prior to packaging for mite mortality evaluations and sensory testing. Mite bioassays, red-legged beetle bioassays and inoculated hams were then shipped to Kansas State University to evaluate mite mortality.

3.2.2.3 Fumigation in Commercial Processing Plant

Two fumigation trials were conducted in a commercial dry cured ham processing plant in the Southeastern United States. The first trial was conducted in a 1000 m^3 aging room. Magnesium phosphide cells were used to produce phosphine gas targeted at 2000 ppm for 48 hrs. Ten mite bioassays were placed in the center and corners of each aging room. Four hams that were aged for about 3 months from this plant were placed in the aging room during fumigation. Two fumigated hams together with two non-fumigated control hams from the same batch were shipped to Mississippi State University after fumigation for sensory evaluation. Mite bioassays were shipped to Kansas State University to determine mite mortality. The temperature inside the aging room was

26±1°C with 70%-80% relative humidity. The second fumigation trial was conducted in the same processing plant during the winter season when the temperature inside the processing plant was 17°C.Two aging rooms were fumigated at a 2000 ppm target phosphine concentration for 48 h with magnesium phosphide cells. One room (R1) was 1000 m^3 and contained 5000 hams that were infested with ham mites. Another room (R2) was 425 m^3 and contained 2000 infested hams. After fumigation was completed, two hams from each fumigation room plus two non-fumigated control hams were shipped to Mississippi State University for phosphine residue detection and sensory tests.

3.2.3 Determination of Phosphine Residue

3.2.3.1 Standard Curve of Phosphine

Amber gas-tight glass vials (40ml, O.D. 28 mm, 98 mm height, Supelco, Bellefonte, PA) with an open center propylene screw cap and Teflon faced silicone septum (O.D. 22 mm diameter, Supelco, Bellefonte, PA) and a 50 ml gas tight syringe (Hamilton Company, Reno, Nevada, Model Gastight #1050) were used to prepare 0.005 ppm, 0.0075 ppm, 0.01 ppm, 0.025 ppm, and 0.05 ppm phosphine gas from 0.5 ppm standard phosphine. To dilute the 0.5 ppm phosphine to 0.05 ppm, 4 ml air was first taken out from the 40ml amber vial by the gas-tight syringe, and followed by injecting 4ml 0.5ppm phosphine. Other concentrations were prepared the same way by first taking out a certain volume of air from the vial and then injecting the same volume of phosphine using the gas-tight syringe. Three replications of each concentration were made for extraction.

Fibers were exposed in the vial for 30 min at 25°C prior to desorbtion in the injection port of GC-pFPD. Since phosphorous compounds were detected in dry-cured

ham after heating, that had similar retention times to phosphine when using the method for fiber selection, a new gas chromatography method was created. The parameters of this method were: an initial oven temperature of 40°C with a 2 min holding time, followed by a 5°C/min ramp rate to 70°C with a hold time for 1 min prior to a 40°C/min ramp rate to 125°C with a hold time of 5 min. The same SPME fiber, extraction time and gas chromatography program were applied to determine if phosphine residue was present in fumigated hams.

3.2.3.2 Residue Detection in Dry-Cured Hams Fumigated with Phosphine

For ham slices that were fumigated under laboratory conditions, two samples from each ham (approximately 10g of sample in gas-tight glass vials) that were aerated 1 min after fumigation, two days after fumigation, and four days after fumigation were evaluated using the GC-pFPD method. For hams that were fumigated in the commercial processing plant, six hams from the second fumigation trial were tested for phosphine residue: two hams from each of the two fumigated rooms and two control hams. One raw sample and one cooked sample were tested from each ham. For cooked samples, ham slices were oven baked at 177°C to reach an internal temperature of 71°C. For both raw and cooked samples, 10 grams of sample were placed into 40ml gas-tight amber glass vials and extracted with an SPME fiber (2cm 50/30 DVB/CAR/PDMS) for 30 min. The extraction temperature was at 25ºC for raw samples and 70ºC for cooked samples.

3.2.4 Mite Mortality

This section was conducted by Dr. Thomas W. Phillips's lab in Dept. of Entomology at Kansas State University. Hams inoculated with mites and mite bioassay

jars were shipped to Kansas State overnight and the number of live mites were counted using a binocular microscope (Olympus, Model SZX10, Center Valley, Pennsylvania). Dead mites don't move when touched with a fine one hair brush. Mite mortalities were evaluated two weeks post fumigation. A mixture of life stages, including eggs laid before the fumigation was assumed in the bioassay jars, so the two weeks allows for egg hatch and other development.

3.2.5 Sensory Evaluation: Difference from Control Test

For the fumigation trials conducted in the simulated aging houses, five hams from each shipping container were cut into 1.27 cm thick slices, vacuumed packaged, and stored at 4°C until they were cooked for sensory panels. For hams fumigated in the commercial processing plant in the first trial, two hams from the fumigated aging room and two non-fumigated control hams were tested. In the second trial, two hams from each of the two fumigated aging rooms and two control hams were tested. For difference from control tests, eight panelists with an average of 50 h of previous experience evaluating meat products evaluated the ham 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. Ham slices were wrapped in aluminum foil bags and oven baked at 177°C to an internal temperature of 71˚C. Upon serving, ham slices were cut into 2.5 cm× 2.5 cm square pieces and placed into 29.5 ml clear plastic containers (Sweetheart Cup Co., Owing Mills, MD) that were coded with 3 digit random numbers. Samples were presented to the panelists in randomized order to account for bias. Water, apple juice, unsalted crackers, and expectorant cups were provided to panelists who were seated in separate booths during each panel.

3.2.6 Statistical Analysis

A randomized complete block design with three replications was used to determine if there was a difference between fumigated and non-fumigated hams ($P<0.05$) for each fumigation trial. Linear regression was used to make the standard curve for phosphine residue quantification; three replications for each concentration were measured.

3.3 Results and Discussion

3.3.1 Effect of SPME Fiber Types and Extraction Time on Extraction Efficacy of Phosphine

The peak area representing the amount of 0.5 ppm phosphine that was absorbed by different SPME fibers and varying extraction times are shown in Figure 3.1. The amount of phosphine extracted by the 65µm PDMS/DVB, 75 µm CAR/PDMS and 85 µm CAR/PDMS increased slowly and slightly with increasing extraction time. The signal peak area increased progressively from 5 min to 25 min for 2cm 50/30 µm DVB/CAR/PDMS and became stable between 25min and 30min. An extraction time of 30 min with the 2cm 50/30 µm DVB/CAR/PDMS was selected for phosphine residue detection because of the larger peak area ($P<0.05$) at all extraction times from 10 to 30 min when compared to the other SPME fibers. The 50/30 µm DVB/CAR/PDMS stationary phase was the only fiber tested with a 2 cm length which translates to a greater number of pores to trap phosphine molecules when compared to the other fibers since they were all 1 cm in length. For the CAR/PDMS fibers, the 85µm extracted more phosphine than the 75µm fiber, which is likely due to its thicker coating. Ren and Padovan (2010) evaluated the efficacy of using 10µm PDMS, 85µm CAR/PDMS, 75µm CAR/PDMS, and 65µm PDMS/DVB to extract phosphine from wheat. The 85µm

CAR/PDMS fiber with an extraction time of 20 min was selected for the analysis since it had a greater peak area for phosphine than the other fibers. The non-polar PDMS fiber was not able to extract phosphine and DVB/CAR/PDMS was not tested in this study (Ren & Padovan, 2010).

3.3.2 External Standard Curve for Phosphine Residue Determination

Since the legal phosphine residue in processed food products is 0.01 ppm (40 CFR 180.225), a series of 0.005 ppm, 0.0075 ppm, 0.01 ppm, 0.025 ppm, and 0.05 ppm were selected to make the standard curve for phosphine residue determination. The standard curve was linear from 0.005 ppm to 0.05ppm with an R^2>0.99 (Figure 3.2). The limit of detection (LOD) was calculated as (3×standard error)/slope (Schilling, 2014). In this trial, the standard error was 50.56uVols and the slope was 43282.8uVols/ppm. The LOD was calculated as 0.0035ppm for this method. According to the standard curve, any fumigated ham sample that exceeds 290 uVols peak area (Figure 3.3) would be beyond the legal residue requirement for processed food products.

3.3.3 Laboratory Fumigation Trials with Phosphine

After 24 h of fumigation, the phosphine concentration inside the three jars labeled as J1, J2 and J3 were 986.0 ppm, 965.4 ppm, and 859.4 ppm, respectively. After 48 h, the average concentrations were: 939.6 ppm, 862.5 ppm, and 706.4 ppm, respectively. A hole was punctured in the middle of the rubber part inside the metal lid before fumigation to hang the ham piece inside the jar with an iron wire, which might have caused slight leakage. After 1 min of aeration in open air, the residues were too high to determine the actual concentration with the standard curve that was developed (Figure 3.4). However, it

was evident that these pieces of ham contained concentrations of phosphine greater than the legal limit. Standard curves to determine higher concentrations of phosphine residue were not constructed since 0.01 ppm is the legal limit and any concentration beyond that point would be considered illegal for processed food products. After 2 days in open air, no residue was detected from pieces in J1 and J2. For J3, two out of the three samples contained phosphine residue with an average of 0.02 ppm. After 4 days in open air, no residue was detected. After released into the ambient atmosphere, phosphine will be transformed into harmless levels of phosphoric acid and phosphates by photolytic reactions (Klementz, Heckemüller, Reichmuth, Huyskens-Keil, Büttner, Horn, & Horn, 2005). Results suggested that for ham pieces fumigated under lab conditions at 1000 ppm for 48h, phosphine residue was below the LOD (0.0035 ppm) after 4 days of aeration. Similar results were observed from other fumigation studies. For example, after fumigation of 'Royal Gala' apples at 1274 ppm for 48 h, the residue dropped below 0.01ppm within 24 h at 5°C (Brash, Klementz, Wimalaratne, van Epenhuijsen, Bycroft, Somerfield, & Reichmuth, 2009). For table grapes fumigated at 2533 ppm for 48 h, the residue was 0.01 ppm after 3 days and dropped below the detection limit after 7 days (Klementz, Heckemüller, Reichmuth, Huyskens-Keil, Büttner, Horn, & Horn, 2005).

3.3.4 Fumigation Trials in Simulated Aging Houses

The concentrations inside the shipping containers were monitored with Dräger tubes during the three fumigation trials (Figure 3.5). Phosphine requires a highly sealed enclosure to reach and maintain the target concentration. The maximum concentration of phosphine was achieved between 12 to 36 hours with trial 2 closest to the target concentration. Even though the shipping containers were sealed by professional

personnel from a fumigation company, it was difficult to maintain gas tight conditions and achieve the target concentration of phosphine.

All life stages of red-legged ham beetles were controlled in the first two trials. The mite mortality was 99.8 % in the bioassays at two weeks post fumigation when 2000 ppm phosphine was achieved, but not all eggs in the bioassays were controlled. Thus a longer exposure time (72 h) was carried out in the third trial; however, during the third trial, the minimum ambient temperature dropped to 1°C at night which was too low to achieve successful control for both red-legged beetles and ham mites with phosphine, since the toxicity of phosphine declines with reduced temperature. Longer exposure time is required when the temperature falls to 5°C. In addition, it is not recommended to fumigate with phosphine at temperatures below 5°C (Bond, 1984). For example, to achieve 100% control of all life stages of tobacco moth through phosphine fumigation, the minimum exposure time was suggested to be 6 days at 16-20°C with 300 ppm phosphine. If fumigated at a temperature greater than 20°C, 4 days of exposure with 200 ppm phosphine was suggested (CORESTA, 2013). The fumigation was conducted during the time of year when the low and high temperatures are generally 15 ºC and 26 ºC, but there was an unusual cold spell that week.

Sensory tests indicated that trained panelists could not determine differences ($P>0.05$) between phosphine treated dry-cured hams and non-fumigated control hams (Table 3.1). As a small and volatile molecule, phosphine penetrates into products and, likewise, dissipates quickly out of products after fumigation (Liu, 2008). Fruits fumigated with phosphine were reported to have mild off-flavor which disappeared after 5-6 days of storage (Horn & Horn, 2004).

3.3.5 Fumigation Trials in Commercial Ham Processing Plant

For the first fumigation trial conducted at the processing plant, the phosphine concentrations were 1060 ppm after 16 h, 1410 ppm after 24 h, and 1200 ppm after 48 h. The mite mortality inside the ten bioassay jars was evaluated two weeks post fumigation and no living mite was detected. Difference from control sensory panels indicated that trained panelists (6-8) could not determine differences ($P>0.05$) between phosphine treated dry cured hams and non-fumigated hams (Table 3.1).

In the second trial, a small leak was detected from aging room 2 (R2) on the backside of the building and the phosphine concentration in R2 was 700ppm on average. Compared with R2, the concentration in room 1 (R1) was 1500ppm on average. There was no visible mite activity in R1 and R2 for approximately one month post fumigation. However, live mites were detected in the mite bioassay jars from both rooms 3 weeks post fumigation. One reason might be the ambient temperature was not ideal for phosphine fumigation as lower temperatures will need longer exposure time. New techniques have been developed to assist fumigation at lower temperatures, for example, the VAPORPH3OS® and HORN DILUPHOS SYSTEM® were designed to fumigate with pure phosphine between -1.5 ºC and 15ºC at 700-3500 ppm for fresh fruits and vegetables (Finkelman, Navarro, Navarro, Ashbell, & Glaser, 2012; Horn, 2004). Similar devices might be developed for dry-cured ham fumigations. Difference from control tests indicated that there was no difference ($P>0.05$) between hams fumigated in R1 and control hams, and between hams fumigated in R1 and R2. However, compared with the negative control which was rated as ‘slightly different’ from control, hams fumigated in R2 were evaluated as ‘moderately different’ from control. Panelists commented that hams

from R2 had “less ham flavor and were less salty than the control” which was highly likely due to the aging time difference since control hams and hams from R1 had been aged longer than hams in R2 (Table 3.1).

One drawback of phosphine fumigation is corrosion to certain metals such as copper, silver and gold (Phillips, Thoms, DeMark, & Walse, 2012). Phosphoric acid was produced when generating phosphine from magnesium/aluminum phosphide. A mild acid will be formed on the copper surface that causes corrosion when phosphoric acid and sufficient moisture are combined (Mueller, 1994). The corrosion rate increases with increased phosphine concentration, longer exposure time and/or higher relative humidity (Brigham, 1999). After the first fumigation trial, the electrical switches to the fans in the aging room were corroded by phosphine and had to be replaced. To investigate how corrosive phosphine was to machinery with copper, copper fittings were purchased from a local hardware store and placed in the aging room during the second fumigation trial (Figure 3.6). After fumigation, the copper fittings were severely corroded. All light switches and ceiling fans were no longer functional and more than $28,000 in electrical damage was discovered after fumigation. This is why phosphine fumigation is rarely used in buildings with extensive electrical systems such as food plants. Extreme caution is needed for future fumigation trials to protect electronic devices from corrosion. If the susceptible electronic apparatus cannot be removed from the fumigation area, protective action such as lubricating oil spray or a layer of paraffin may be applied to the copper materials (Bond, 1984).

3.4 Conclusions

Though phosphine was successful at controlling mites under laboratory conditions and had limited success at controlling mites under commercial conditions, it was highly corrosive in the ham plant and significant precautions would ne needed if used in plant operations. In addition, an analytical method was successfully developed to determine the concentration of phosphine, and it was demonstrated that phosphine levels in dry cured ham was not detected 4 days post-fumigation.

Table 3.1 Difference from Control Test results for phosphine fumigated ham (1-no difference, 2-slight difference, 3-moderate difference, 4-large difference, 5-very large difference).

Treatment		Mean
Fumigation in Simulated Aging houses	Negative control	2.0[a]
	Low phosphine (2000 ppm)	2.3[a]
	High phosphine (2500 ppm)	2.3[a]
First Fumigation in Processing Plant	Negative control	2.0[a]
	Phosphine (2000 ppm)	2.1[a]
Second Fumigation in Processing Plant	Negative Control	2.1[a]
	Aging Room 1 (2000 ppm)	2.6[ab]
	Aging Room 2 (2000 ppm)	3.1[b]

[1]Means with same letter are not significantly different ($p>0.05$).

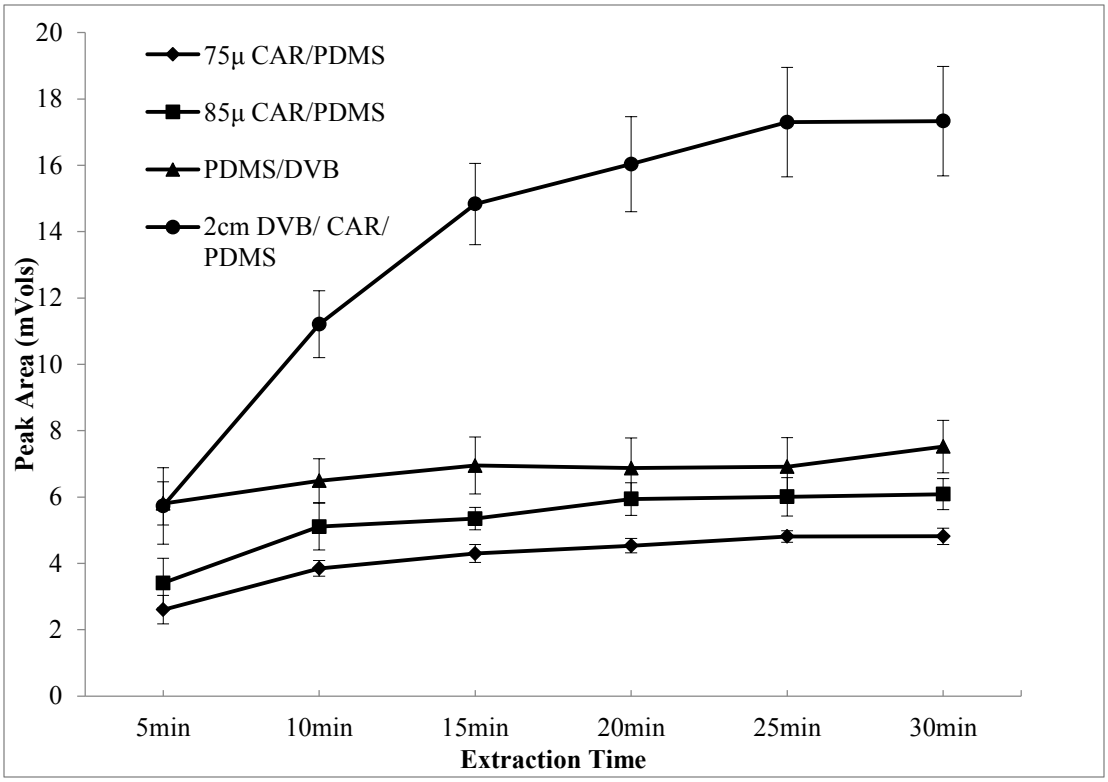

Figure 3.1 Effect of SPME fiber types (PDMS/DVB, 75 µm CAR/PDMS, 85 µm CAR/ PDMS, and 2cm 50/30 µm DVB/CAR/PDMS) and extraction time on the extraction efficacy of 0.5ppm phosphine.

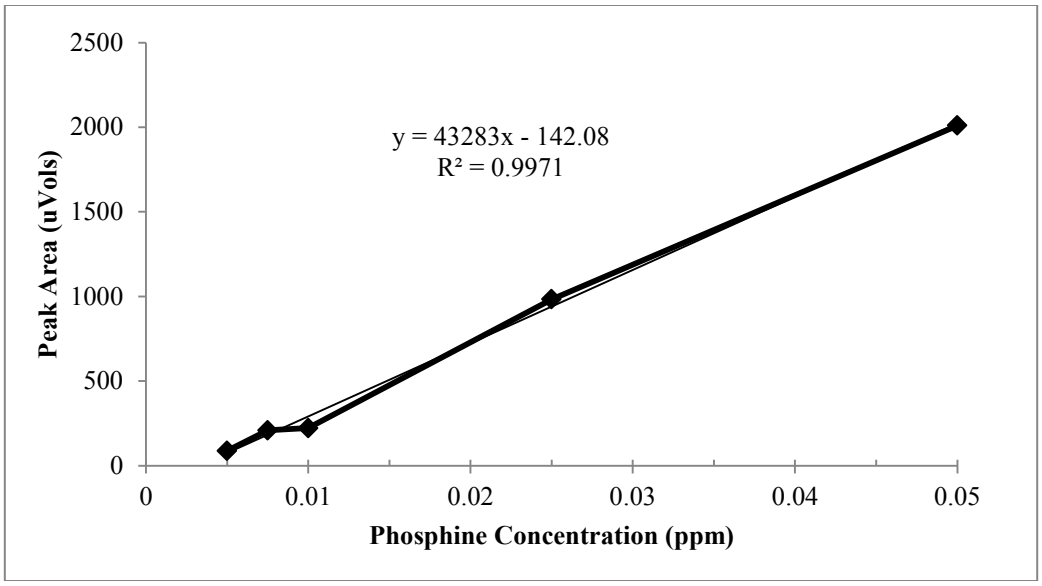

Figure 3.2 Standard curve (0 ppm, 0.005ppm, 0.0075ppm, 0.01ppm, 0.025ppm, and 0.05ppm phosphine standards) for phosphine residue determination with gas chromatography pulsed flame photometric detector.

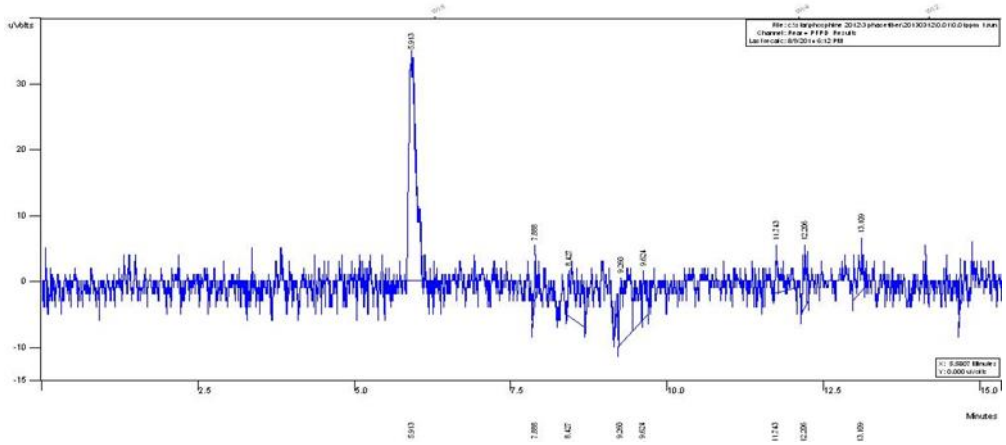

Figure 3.3 Gas chromatography of phosphine using a 2cm 50/30 µm DVB/CAR/PDMS after 30 min extraction at 0.01 ppm level of phosphine (Unit: uVols).

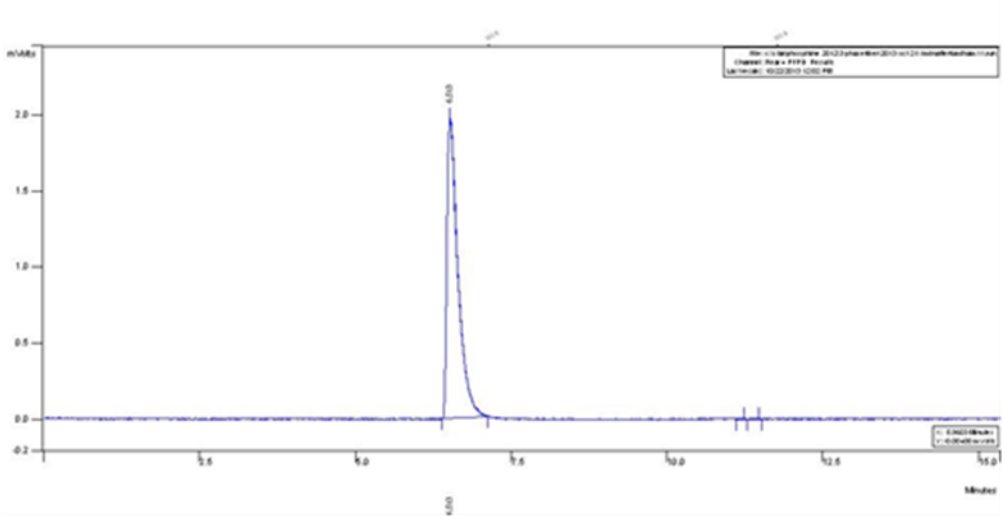

Figure 3.4 Phosphine residue of ham piece aerated for 1 min after fumigation at 1000 ppm for 48 h (Unit: mVols).

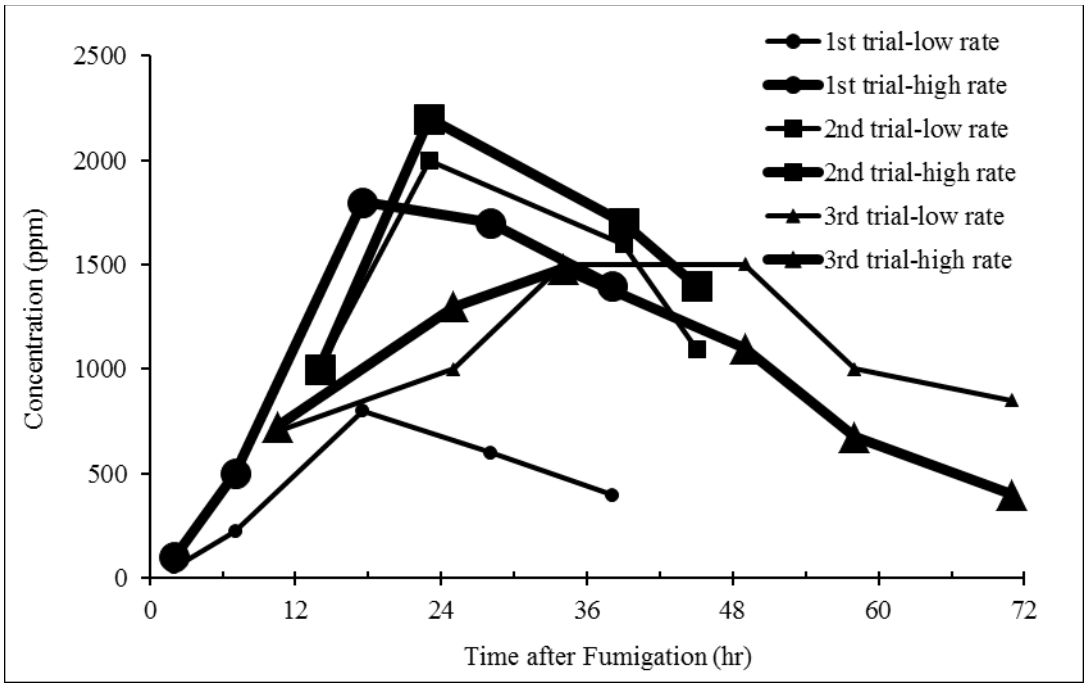

Figure 3.5 Phosphine concentration inside simulated ham aging houses during fumigation.

Figure 3.6 Copper fittings placed in two rooms at a commercial ham processing facility during a fumigation trial: top fittings-untreated control room; bottom fittings-phosphine fumigated room.

CHAPTER IV

DEVELOPMENT OF FOOD-GRADE COATINGS FOR DRY-CURED HAM

4.1 Introduction

Many different types of dry-cured hams are currently produced around the world. Some of the most popular dry-cured hams are Iberian and Serrano ham from Spain, Corsican ham from France, country style ham from the United States, Westphalia ham from Germany, and Jing Hua ham from China. Aging, also known as ripening, is the processing step that develops the unique and characteristic aroma and flavor of dry-cured ham. Aging conditions are different based on the type of ham, and the length of the aging process varies from 3 to 36 months (Toldrá, 2010). *Tyrophagus putrescentiae*, also known as the mold or cheese mite, is a cosmopolitan species that infests stored food products such as grains, peanuts, cheese, cotton seed, and dry-cured ham. Females kept on wheat germ or yeast at 20ºC and 85% RH are able to lay up to 500 eggs during their life span. At 20ºC and 85%RH, depending on the type of food available, the mold mites complete one generation in 10 to 24 days (Boczek, 1991). Dry-cured ham aging temperature usually ranges between 16 °C and 25 ºC in Europe, and the relative humidity usually ranges between 65% and 80% (Toldrá, 2010). In the United States, the aging temperatures are higher, often greater than 28 °C (Rentfrow, Chaplin, & Suman, 2012). Dry-cured ham is very susceptible to mite infestations after 4-6 months of aging and the environmental conditions where hams are aged also favor mite growth and reproduction

(Rentfrow, Hanson, Schilling, & Mikel, 2008). Mold mites have been reported as a problem for dry-cured ham both in Spain (Sánchez-Ramos & Castañera, 2000) and in the United States (Rentfrow, Chaplin, & Suman, 2012).

Methyl bromide has been used to fumigate commodities and buildings worldwide since the 1930s (Fields & White, 2002) and is the only known fumigant that is effective at controlling ham mite infestations as of 2013 (EPA, 2013). In 1992, methyl bromide was listed as an ozone depleting substance under the Montreal Protocol, in which all developed countries agreed to reduce the amount of their application of methyl bromide by 2005 (TEAP, 2000). Since 2004, critical use exemptions have been granted in developed countries on a yearly basis if a technical and economically feasible alternative with acceptable environmental and health effects was not available. A critical use exemption of 3,240 kg has been approved for dry cured pork products in the United States in 2015 (EPA, 2013). Exploring potential alternatives to control mite infestations is very important for the economic viability of the dry-cured ham industry in US.

Potential alternatives for methyl bromide fumigation include fumigants such as phosphine; physical control methods such as modified atmosphere; pesticides and bioactive compounds such as Storcide II® and limonene from pine essential oils (Abbar, Zhao, Schilling, & Phillips, 2013; Macchioni, Cioni, Flamini, Morelli, Perrucci, Franceschi, & Ceccarini, 2002; Sánchez-Molinero, García-Regueiro, & Arnau, 2010). Beside the alternatives mentioned above, it has been stated that coating hams with vegetable oils or hot lard is a common practice in Spain to control mite infestations in dry-cured ham (García, 2004). Cured meat has been rubbed with a paste of lard on the surface prior to storage to prevent flies and bacteria for over 100 years (Smith, 1923).

Edible coatings have been applied for different purposes on a variety of food products including fresh fruits and vegetables, confections and meat products. For meat products, edible films and protective coatings have been used to prevent off-flavor due to oxidation, discoloration, quality loss such as shrinkage, and microbial contamination (Ustunol, 2009). For example, film coatings made from k-carrageenan incorporated with ovotransferrin (a protein of avian egg's antimicrobial defense system) and EDTA were applied on fresh chicken breasts and used to inhibit the growth of *E. coli* and total aerobic bacteria during storage (Seol, Lim, Jang, Jo, & Lee, 2009). To be qualified as a coating for dry-cured ham, the compound must 1) be food grade; 2) be able to attach to the ham surface; 3) be able to cover the ham surface evenly; 4) be stable during the aging process; 5) be permeable to water vapor and oxygen; 6) be able to suffocate, kill, and/or repel mites and insects when applied properly; 7) not adversely affect ham flavor; 8) be easily removed after the aging process. The objectives of this research were 1) to evaluate food grade coatings for their efficacy at controlling mite infestations under laboratory conditions; 2) to determine if the coatings that are developed are permeable to oxygen and moisture; 3) to determine if the use of coatings affects the sensory properties of the dry cured ham.

4.2 Materials and Methods

4.2.1 Food-Grade Coating Materials on Ham Cubes

4.2.1.1 Materials

Lard (ConAgra Foods, Omaha, NE), mineral oil (CVS® Pharmacy Inc., Woonsocket, RI), glycerin (Essential Depot, Sebring, FL), propylene glycol (Essential Depot, Sebring, FL), and potassium sorbate (Crosby&Baker Ltd., Westport, MA) were

purchased as coating materials. Ten percent potassium sorbate solution in distilled water was prepared.

4.2.1.2 Ham Preparation

Six dry-cured hams were purchased from a commercial ham plant. From each ham, seven 1.3cm thick slices and five 2.5cm thick slices were obtained. The 2.5cm slices were then cut into 2.5 cubic centimeter cubes for the mite infestation study. Ham slices/cubes were dipped directly into mineral oil, propylene glycol, 10% potassium sorbate solution, and glycerin respectively for 1 minute and allowed to drip on a mesh colander for another minute. Lard was applied directly by rubbing a thin layer to cover the entire area.

For sensory evaluation, five 1.3cm thick slices from each ham were treated with mineral oil, propylene glycol, potassium sorbate, glycerin and lard, respectively. The other two slices from each ham were non-treated control slices. Slices were then vacuum-packed and stored at 4 ºC for further sensory analysis. For mite bioassays, one cube from each ham was randomly selected to treat with mineral oil, propylene glycol, potassium sorbate, glycerin, and lard. Another cube from the ham slices was also randomly selected and freeze-dried until the water activity dropped to 0.65 on the surface and 0.80 inside the cubes. Treated cubes were packaged in zip-lock bags and shipped overnight to Kansas State University, Manhattan, KS for the mite infestation study.

4.2.1.3 Mite Infestation Study

This section was conducted by Dr. Thomas W. Phillips's lab in the Department of Entomology at Kansas State University. Twenty mites (at least 50 percent adult female)

were transferred onto each cube, and the cube was placed in a ventilated, mite-proof 118ml glass container to incubate for 21 days at 27ºC and 70% relative humidity. The lid of the container had a filter paper insert which not only allowed gas exchange but also prevented mites from escaping. Mite populations on ham cubes were counted under the microscope. Counting was stopped when the number reached 500.

4.2.2 Development of Film Coatings with Polysaccharide and Propylene Glycol

4.2.2.1 Materials

Since initial laboratory coating tests indicated that propylene glycol was effective at controlling mite infestations, further studies were carried out to develop film coatings that contained propylene glycol and polysaccharides. This was done to keep the propylene glycol on the ham so that it remains active at controlling mites during aging. Preliminary tests on polysaccharides suggested that 50% propylene glycol was effective at controlling mites with 2 % carrageenan. To develop a polysaccharide gel solution with up to 50% propylene glycol, the following materials were also tested: modified food starch (INSTANT PURE-COTE, Grain Processing Corporation, Muscatine, IA52761), agar (Tic Pretested® Agar RS-100 Powder, TIC Gums, Belcamp, MD 21017), carrageenans (MBF-120i, x, INC., Waldo, ME 04915; MBF-9414, Ingredients Solutions INC., Waldo, ME 04915; Ticgel 795, TIC Gums, Belcamp, MD 21017), propylene glycol alginate (Tica-algin PGA, TIC Gums, Belcamp, MD 21017), methycellulose (TICAGEL® HV Powder, TIC Gums, Belcamp, MD 21017), sodium alginate (TICA-algin® 400 Powder, TIC Gums, Belcamp, MD 21017), and xanthan gum (Pre-hydrated Ticaxan Rapid-3 powder, TIC Gums, Belcamp, MD 21017).

4.2.2.2 Solution Preparation with Polysaccharides and Propylene Glycol

To evaluate how different polysaccharides interact with propylene glycol, combinations were tested (Table 4.1). For cold water soluble polysaccharides, distilled water at room temperature was used. All solutions were made in glass beakers with a magnetic stir bar inside each beaker. Solutions were stirred on magnetic stir plates until homogeneous. For hot water soluble polysaccharides, boiled water was used and the solutions were stirred on hot plates until homogeneous. Metal meat hooks were used to dip ham cubes ($2.5\times2.5\times2.5cm^3$) into the gel solutions. Coated cubes were hung at 24ºC and 50% RH to determine the film-forming abilities of the tested combinations.

4.2.2.3 Ham Preparation for Mite Bioassay

Three sets of dry-cured ham cubes ($2.5\times2.5\times2.5cm^3$) were prepared for three mite bioassays. For the first mite bioassay trial, ham cubes were coated with pure polysaccharides and no propylene glycol. Agar (Tic Pretested®Agar RS-100 Powder, TIC Gums), propylene glycol alginate (Tica-algin® PGA LV Powder, TIC Gums), carrageenan (Ticagel® 795 Powder, TIC Gums), and xanthan gum (Pre-Hydrated®Ticaxan®Rapid-3 Powder, TIC Gums) were tested to evaluate the effectiveness of pure polysaccharide coatings at controlling mites. For the second and third trial, propylene glycol (Essential Depot, Sebring, FL) was combined with polysaccharide solutions (Table 4.2). Xanthan gum was solubilized at room temperature, and other polysaccharides were solubilized in boiling water, heated and stirred until homogenous. The viscosity of the gel solutions increased as the temperature cooled. To maintain a consistent thickness of coatings on the cube surfaces, the temperatures of the dipping solutions were controlled (Table 4.2). Three commercially aged hams were used

during each trial. Two cubes from each ham were dipped in each treatment, which led to 6 cubes per treatment in total.

4.2.2.4 Mite Bioassay

Mite bioassays were conducted at Kansas State University. Twenty large (at least 50 percent adult female) mites were placed on each cube of ham, and the cube was placed in a mite-proof, ventilated glass container and incubated for 2 weeks. The number of live mites was counted with binocular microscope (Olympus, Model SZX10, Center Valley, Pennsylvania). Dead mites don't move when touched with a fine one hair brush.

4.2.3 Film Characterization

4.2.3.1 Film Preparation for Characterization

Propylene glycol alginate (Tica-algin® PGA LV Powder, TIC Gums), carrageenan (Ticagel® 795 Powder, TIC Gums), and xanthan gum (Pre-Hydrated®Ticaxan®Rapid-3 Powder, TIC Gums) were used to form gel solutions with propylene glycol (Essential Depot, Sebring, FL). The combinations were the same as those in the third trial (Table 4.3), with the exception of the pure xanthan gum solution since xanthan gum has very poor film forming capacity at the applied percentage. Solution was poured into a 150×15mm plastic petri dish to form a thin layer of film. To estimate the amount of the gel solution coated on ham per surface area, ham cubes with 2.5cm length on each side were coated and the weight gain per square centimeter was calculated. Based on the results of weight gain per unit area of different treatments and also to maintain the consistency of the amount of polysaccharides on each treatment, the amount of gel solution poured on each petri dish was rounded up to 25 g for all

treatments. The films were dried out at 24±0.5ºC and 50%±2% RH until the weight of films remained constant.

4.2.3.2 Film Thickness

Film thickness was measured using a digital micrometer (Fowler®, Model: 54-815-001-2, Newton, MA) with 0.002 mm accuracy. Three films were measured for each treatment and two measurements were taken from each film.

4.2.3.3 Water Vapor Permeability

Water Vapor Permeability (WVP) was tested according to ASTM method E96-95 (1995) with some modifications (Ghanbarzadeh, Almasi, & Entezami, 2011). Gas-tight amber glass vials (40 ml, o.d. 28 × 98 mm height) with propylene screw caps and Teflon faced silicone septa (o.d. 22 mm) were used to determine the WVP of films. Films were cut into round discs that were the same size and shape as the septa. On each septum, a 14 mm o.d. hole was cut through at the center. The test film was placed in between the screw cap and the septum. The cap was tightly screwed to the vial so that the only water vapor exchange pathway between the inside and outside of the vial was through the 14 mm o.d. film area. Three grams of anhydrous $CaSO_4$ (Cat No: AC217525000, Fisher Scientific, USA) was added in each cup to maintain 0% RH inside the cup. Cups were then placed in a desiccator containing saturated K_2SO_4 solution so that the RH inside the desiccator was maintained at 97% at 25ºC. Cups were weighed every 2 h for the first day and then every 12 h for another day. Changes of weight were recorded as a function of time. Slopes (weight vs. time) were calculated by linear regression. Water vapor transmission rate

(WVTR) was calculated as slope (g/h) divided by the transfer area (m^2). WVP ($gPa^{-1}h^{-1}m^{-1}$) was calculated as

$$WVP = \frac{WVTR \times T}{P(R1\text{-}R2)} \quad (4.1)$$

where T is the film thickness (m). P is the saturation vapor pressure of water (Pa) at test temperature. R1 is the RH inside the desiccator and R2 is the RH inside the vial. P(R1-R2) is the driving force, and under the RH settings of this experiment at 25ºC, the driving force was 3074 Pa.

4.2.4 Sensory Evaluation

Ham slices treated with lard, mineral oil, glycerin, propylene glycol, and potassium sorbate were evaluated (as described in section 4.2.1.2). Ham slices dipped in distilled water were considered the negative control. Coatings on ham slices were washed off with tap water at room temperature before cooking. Ham slices were wrapped in aluminum foil pouches and oven baked at 177°C to an internal temperature of 71˚C. Upon serving, ham slices were cut into 2.5× 2.5 cm square pieces and placed into 29.5 ml clear plastic containers that were coded with 3-digit random numbers. Samples were presented to the panelists in a randomized order. Water, apple juice, unsalted crackers, and expectorant cups were provided to panelists who were seated in separate booths during each panel. 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.

4.2.5 Statistical Analysis

A randomized complete block design with three replications was used to determine if trained panelists (n= 6-8) could detect a difference between coated and non-coated hams ($P<0.05$). A completely randomized design with three replications was used to determine the effect of different treatments on ham mite mortality. When significant differences ($P<0.05$) occurred among treatments, Tukey's Honestly Significant Difference Test ($P<0.05$) was used to separate treatment means.

4.3 Results and Discussion

4.3.1 Mite Mortality and Sensory Test of Food-Grade Coating Materials

The mean numbers of live mites on ham cubes that were treated with different food-grade materials and incubated for 3 weeks are shown in Table 4.3. Six cubes were freeze-dried to determine the effect of water activity on mite development. No differences existed ($P>0.05$) between the control, freeze dried, and glycerine treatments. This indicates that glycerine ($P>0.05$) did not inhibit mite reproduction. All of these treatments increased from 20 to greater than 200 mites after 3 weeks of inoculation. Potassium sorbate and mineral oil treatments had fewer mites ($P<0.05$) than the control, but their mite populations had grown from 20 to 77 and 94, respectively, which indicates that mineral oils and potassium sorbate were not effective at controlling mites (Table 4.3). Lard and propylene glycol had fewer mites ($P<0.05$) than the control, freeze-dried and glycerine treatments. Since lard and propylene glycol had an average of 2 mites on the ham pieces after 3 weeks of incubation, it was evident that these 2 treatments were effective at controlling mites at the benchtop level. Lard could likely be used to control mites once the product has completed the aging process since it is not permeable to

oxygen or moisture. However, it could not be used until after aging is complete, which inhibits its usability during commercial settings.

For sensory tests, control and coated ham slices were vacuum-packed and stored at 4ºC for two months and then washed off prior to sensory panels. No difference ($P>0.05$) was detected in sensory characteristics between control ham slices and slices treated with food grade ingredients (Table 4.3). A negative control was applied to setup the baseline for the determination of difference. Compared with coating a whole ham, coating ham slices exposed much more muscle area to the coating materials. If no difference was detected from coating ham slices, it is logical that the same coating materials would not affect the sensory profile when coating a whole ham. In addition, propylene glycol has been used as a humectant in soft-moist dog foods and has been reported to be effective at controlling mite infestations (Aldrich, 2014; Phillips, 2011).

4.3.2 Initial Coating Tests with Polysaccharides and Propylene Glycol

Since propylene glycol was effective at controlling mites and is permeable to moisture and oxygen when used in coatings (Sothornvit & Krochta, 2000), it was selected for use with different polysaccharides to develop a gel solution with desired viscosity to form a consistent film coating on the ham surface. Polysaccharides/propylene glycol combinations were tested as shown in Table 4.1. Since Abbar et al, (2013) showed that 50 % propylene glycol mixed with water was effective at controlling mites (Abbar, Zhao, Schilling, & Phillips, 2013); polysaccharides from Table 4.1 were tested with up to 50 % propylene glycol. Inclusion of high percentages of propylene glycol affected the forming abilities of the polysaccharides that were evaluated. Starch, carrageenan, sodium alginate (with added Ca^{2+}) formed strong gels with pure water; however, with 50% propylene

glycol, the polysaccharides either did not gel or formed a very weak gel. On the contrary, propylene glycol alginate formed a weak gel with pure water, but its gel forming capacity increased as the percentage propylene glycol increased. When propylene glycol alginate and carrageenan were combined, a very consistent film coating was formed on the ham surface with 50% propylene glycol. Xanthan gum formed viscous and consistent gels with and without the addition of propylene glycol.

Based on results from the initial dipping tests in Table 4.1, selected polysaccharides were mixed with either 0 or 50% propylene glycol for initial mite mortality tests (first and second trials in Table 4.2). All polysaccharide coatings with 0% propylene glycol had fewer mites ($P<0.05$) than the control treatment, but there was no difference ($P>0.05$) in the number of mites between these coating treatments (Table 4.4). All coatings with 50% propylene glycol had fewer mites ($P<0.05$) than the Control and Agar treatments. In addition, no mites were detected in any coating treatments with 50% propylene glycol (Table 4.4).

4.3.3 Coatings Developed with Polysaccharides and Propylene Glycol

Since an objective of this research was to find an effective and economic alternative for methyl bromide, the cost of the food grade coatings should be minimized as long as the effectiveness at controlling mites is not diminished. Since the numerical number of mites were less in the xanthan gum and PGA + CG treatments (Table 4.4), these 2 treatments were chosen for further testing to minimize the propylene glycol concentration and thus reduce application cost. Xanthan gum (XG 1%) and propylene glycol alginate (PGA 1%)+carrageenan (CG 1%) were selected to conduct dipping tests

for their capability to form thick and consistent gel solutions with 0 - 50% propylene glycol (Table 4.5).

Similar to the results in Table 4.4, the 2 coating treatments with 0 % propylene glycol were effective ($P<0.05$) at reducing the mite populations when compared to the control. When 10 % PG was added to the PGA+CG treatment, the mite population was further reduced ($P<0.05$) to almost non-detectable levels at 2. This indicates that propylene glycol could be added at concentrations as low as 10 % and control mites on ham cubes. All treatments with XG or PCA+CG with 20 % PG yielded no detectable mites and had fewer mites ($P<0.05$) than the control and the coated treatments with no propylene glycol. This indicates that XG was effective at controlling mites on ham cubes at concentrations of 20 % PG and greater. These results infer that tests should be scaled up in simulated aging houses and commercial dry cured ham facilities.

4.3.4 Film Properties

4.3.4.1 Film Appearance

As a humectant, PG keeps the films from drying out fast due to its two hydroxyl groups that attract and retain water molecules. The more PG added in the solution, the longer it took for the films to reach a consistent weight at 25ºC and 50% RH. After dried out, films with PG all wrinkled to some extent as seen in Figure 4.1. Three possible explanations are: 1) different parts of the film dried at slightly different rates during drying because of the slight difference of PG distribution; 2) randomness of breaking/reforming of hydrogen bonds due to a slightly different surrounding environment; 3) PG has a slight effect on the polymer rearrangement of polysaccharides during drying. As a processing aid, the food grade coating should be removed from the

ham surface before packaging for distribution. The coatings developed in this study could be easily peeled off from ham cubes as seen in Figure 4.2. In addition, they could also be washed off as well.

4.3.4.2 Thickness

The thickness of films made from xanthan gum and propylene glycol alginate + carrageenan increased ($P<0.05$) as the percentage of propylene glycol increased (Table 4.6). The XG treatment with 20 and 30 % PG were thicker than XG with 10 % PG, but were not different ($P>0.05$) from one another. XG with 40 % PG was thicker than the 10 and 20 % PG treatments but not different from the 30 % PG treatment. XG with 50 % PG was thicker ($P<0.05$) than the 0, 10, 20, and 30 % PG treatments. Similar to the results for XG, the thickness of the PGA + CG films increased linearly ($P<0.05$) as PG level increased. The PGA+CG+50 % PG was thicker than all other treatments, the 40 % PG treatment was thicker than the 0, 10, 20, and 30 % treatments, the 30 % PG treatment was thicker than all percentages below it, and the 0 % treatment was less thick than the 10 and 20 % PG treatments. One reason for increased thickness could be due to shrinkage since increasing PG concentration led to increased shrinking during drying (Figure 4.1).

4.3.4.3 Water Vapor Permeability

Water vapor permeability (WVP) for both films made form xanthan gum and propylene glycol alginate + carrageenan increased with increasing percentage of propylene glycol (Table 4.6). There was a linear and quadratic increase ($P<0.05$) in WVP for xanthan gum as propylene glycol concentration increased. The WVP was greater in PGA +CG when compared to XG. In addition, there was a linear, quadratic, and cubic

increase in WVP as propylene glycol percentage increased from 0 to 50 %. All films were permeable to water, which allows moisture loss during aging which is important for the preservation of the ham. Propylene glycol is the functional ingredient in the film coatings for controlling mites. However, it can also function as a plasticizer to reduce the brittleness of films. When used as a plasticizer, the amount of propylene glycol added to the solution is usually between 10% and 60% by weight of the polysaccharide (Skurtys, Acevedo, Pedreschi, Enrione, Osorio, & Aguilera, 2010). Generally, the addition of plasticizers to polysaccharide films increases film permeability to gas and water vapor (Alves, Costa, & Coelhoso, 2010; Mali, Grossmann, García, Martino, & Zaritzky, 2004; Rao, Kanatt, Chawla, & Sharma, 2010; Skurtys, Acevedo, Pedreschi, Enrione, Osorio, & Aguilera, 2010), similar to what was seen in the current research. It's difficult to compare WVP of films measured by different researchers as the American Society for Testing and Materials (ASTM) published many methods to measure permeability and films were measured under different ambient conditions and expressed with different units. The WVP of films made of low density polyethylene (LPDE) was 0.031 g·mm/m^2·d·kPa when measured at 28ºC and 0-100% RH gradient (Shellhammer & Krochta, 1997). After converting the unit from g·mm/m^2·d·kPa to g/Pa·h·m, the WVP of the LPDE film became 1.3×10^{-9} g/Pa·h·m, which is much lower than the WVP of films made from XG and PGA +CG (Table 4.6).

American dry cured ham products need to lose at least 18% of their original weight during the production process. Therefore, the WVP of the film coatings must be considered when choosing a coating for dry-cured ham. A preliminary test on WVP was carried out by coating whole hams with different coatings and the weight loss of each

ham was recorded after 48 days in simulated aging houses. The coating treatments were: control, 100% propylene glycol, 2% Carrageenan+50% propylene glycol, hot lard dip, and diatomaceous earth. Six hams were treated for each treatment. Compared with control hams which had an average of 7.4% weight loss after 48 days, hams treated with 2% carrageenan+50% propylene glycol lost 6.4% of weight. Hams coated with a thin layer of lard lost 5.3% of their original weight. Hams rubbed with a thin layer of diatomaceous earth lost 6.8% of their original weight.

4.4 Conclusions

Coatings made from propylene glycol, xanthan gum and carrageenan + propylene glycol alginate were effective at controlling mite infestations under lab conditions. In addition, these coatings were permeable to moisture, which is important for the aging process. Further research will be conducted on scaling up these coatings to both experimental (mite inoculated hams) and commercial treatment (natural conditions) of whole dry cured hams. In addition, sensory testing will be conducted to determine if coating whole hams impacts the sensory properties of the ham.

Table 4.1 Combinations of different polysaccharides and propylene glycol (PG) at different ratios for coating tests (w/w).

Polysaccharide	%	with	PG	Water	Heat
PGA	1%	n/a	5%	94%	no
PGA	1%	n/a	10%	89%	no
PGA	1%	n/a	20%	79%	no
PGA	1%	n/a	30%	69%	no
PGA	1%	n/a	40%	59%	no
PGA	1%	n/a	50%	49%	no
PGA	2%	n/a	10%	88%	no
PGA	2%	n/a	20%	78%	no
PGA	2%	n/a	30%	68%	no
PGA	2%	n/a	40%	58%	no
PGA	2%	n/a	50%	48%	no
PGA	0.5%	1% ST	50%	48.5%	yes
PGA	1%	1% ST	50%	48%	yes
PGA	2%	2% ST	50%	46%	yes
PGA	1%	0.5 % CG	50%	48.5%	yes
PGA	1%	1% CG	50%	48%	yes
ST	1%	n/a	50%	49%	yes
ST	2%	n/a	50%	48%	yes
ST	4%	n/a	50%	46%	yes
CG	1%	n/a	10%	89%	yes
CG	1%	n/a	30%	69%	yes
CG	2%	n/a	15%	83%	yes
CG	2%	n/a	30%	68%	yes
CG	2%	n/a	50%	48%	yes
CG	3%	n/a	30%	67%	yes
CG	3%	n/a	50%	47%	yes
MC	1%	n/a	50%	49%	yes
MC	2%	n/a	50%	48%	yes
MC	3%	n/a	50%	47%	yes
XG	1%	n/a	10%	89%	no
XG	1%	n/a	30%	69%	no
XG	1%	n/a	50%	49%	no
XG	2%	n/a	50%	48%	no
SA	1%	n/a	50%	49%	no
SA	2%	n/a	50%	48%	no
Agar	1%	n/a	50%	49%	yes
Agar	2%	n/a	50%	48%	yes

[1]PGA: propylene glycol alginate, ST: starch, CG: carrageenan, MC: methyl cellulose, XG: xanthan gum, SA: sodium alginate.

Table 4.2 Polysaccharides and propylene glycol (PG) treatment combinations (w/w) and dipping temperatures for dry-cured ham cubes.

	First Trial		
Polysacchrides	PG	Water	Dipping Temp
XG 1%	n/a	99%	Rm Temp
Agar 2%	n/a	98%	40ºC
PGA 2%	n/a	98%	60ºC
PGA 1%+CG 1%	n/a	98%	60ºC
Control	n/a	100%	Rm Temp
	Second Trial		
Polysacchrides	PG	Water	Dipping Temp
XG 1%	50%	49%	Rm Temp
Agar 2%	50%	48%	30ºC
PGA 2%	50%	48%	60ºC
PGA 1%+CG 1%	50%	48%	60ºC
Control	n/a	100%	Rm Temp
	Third Trial		
Polysacchrides	PG	Water	Dipping Temp
XG 1%	n/a	99%	Rm Temp
XG 1%	10%	89%	Rm Temp
XG 1%	20%	79%	Rm Temp
XG 1%	30%	69%	Rm Temp
XG 1%	50%	49%	Rm Temp
PGA 1%+CG 1%	n/a	98%	28ºC
PGA 1%+CG 1%	10%	88%	30ºC
PGA 1%+CG 1%	20%	78%	35ºC
PGA 1%+CG 1%	30%	68%	40ºC
PGA 1%+CG 1%	50%	48%	60ºC

[1]PGA: propylene glycol alginate, CG: carrageenan, XG: xanthan gum.

Table 4.3 Mean number of mites on inoculated ham cubes (20 mites/cube) after 3 weeks incubation and difference from control sensory test results of 1.3cm ham slices treated with different food grade coatings after 8 weeks.

Treatment	Mite		Sensory	
	Mean	SE	Mean	SE
Control	336[a]	53.3	n.a.	n.a.
Freeze dried	236[ab]	37.9	n.a.	n.a.
100% Glycerin	219[abc]	48.2	2.0[a]	0.13
100% Mineral Oil	94[bcd]	29.4	2.1[a]	0.14
10% Potassium Sorbate	77[cd]	35.8	1.8[a]	0.13
Lard	2[d]	1.8	1.6[a]	0.15
100% Propylene Glycol	2[d]	0.7	2.1[a]	0.12
Negative Control	n.a.	n.a.	2.0[a]	0.13

[1]Means with same letter within each row are not significantly different ($p>0.05$) on Tukey's test at 5% significance level.
[2]Scale for sensory evaluation: 1-no difference, 2-slight difference, 3-moderate difference, 4-large difference, 5-very large difference.

Table 4.4 Mean number of mites on inoculated ham cubes (20 mites/cube) coated with different polysaccharides and propylene glycol (PG) combinations after 2 weeks incubation.

Polychariddes	PG	Mean	SE
Control	0%	274[a]	52.6
Agar (2%)	0%	111[b]	18.8
PGA (2%)	0%	55[bc]	7.9
XG (1%)	0%	29[bc]	4.2
PGA (1%) + CG (1%)	0%	28[bc]	5.5
Agar (2%)	50%	0[c]	0
PGA (2%)	50%	0[c]	0
XG (1%)	50%	0[c]	0
PGA (1%) + CG (1%)	50%	0[c]	0

[1]PGA: propylene glycol alginate, CG: carrageenan, XG: xanthan gum
[2]Means with same letter within each row are not significantly different ($p>0.05$) on Tukey's test at 5% significance level.

Table 4.5 Mean number of mites on ham cubes (20 mites inoculated/cube) coated with polysaccharides and different percentage of propylene glycol (PG) after 2 weeks incubation.

Polysaccharides	PG	Mite	
		Mean	SE
Control	0%	476[a]	48.7
PGA (1%) + CG (1%)	0%	186[b]	45.2
XG (1%)	0%	155[b]	54.1
XG (1%)	10%	70[bc]	48.2
PGA (1%) + CG (1%)	10%	2[c]	0.5
XG (1%)	20%	0[c]	0
PGA (1%) + CG (1%)	20%	0[c]	0
XG (1%)	30%	0[c]	0
PGA (1%) + CG (1%)	30%	0[c]	0
XG (1%)	50%	0[c]	0
PGA (1%) + CG (1%)	50%	0[c]	0

[1]PGA: propylene glycol alginate, CG: carrageenan, XG: xanthan gum
[2]Means with same letter within each row are not significantly different ($p>0.05$) on Tukey's test at 5% significance level.

Table 4.6 Thickness and water vapor permeability (WVP) of films made from 1% xanthan gum (XG), 1% propylene glycol alginate (PGA)+1% carrageenan (CG), and different percentages of propylene glycol (PG).

PG	1% XG				PGA 1%+CG 1%			
	Thickness (mm)		WVP (10^{-7} g/Pa·h·m)		Thickness (mm)		WVP (10^{-7} g/Pa·h·m)	
	mean	se	mean	se	mean	se	mean	se
0%	n.a	n.a.	n.a.	n.a.	0.026^{e}	0.00040	2.07^{e}	0.010
10%	0.013^{d}	0.00040	1.14^{e}	0.017	0.028^{d}	0.00022	2.25^{de}	0.021
20%	0.016^{c}	0.00037	1.40^{d}	0.001	0.030^{d}	0.00058	2.42^{cd}	0.010
30%	0.018^{bc}	0.00043	1.57^{c}	0.036	0.032^{c}	0.00070	2.60^{c}	0.105
40%	0.019^{ab}	0.00050	1.68^{b}	0.019	0.036^{b}	0.00079	2.96^{b}	0.055
50%	0.020^{a}	0.00060	1.77^{a}	0.039	0.045^{a}	0.00076	3.47^{a}	0.107

[1]Means with same letter within each row are not significantly different ($p>0.05$) on Tukey's test at 5% significance level.

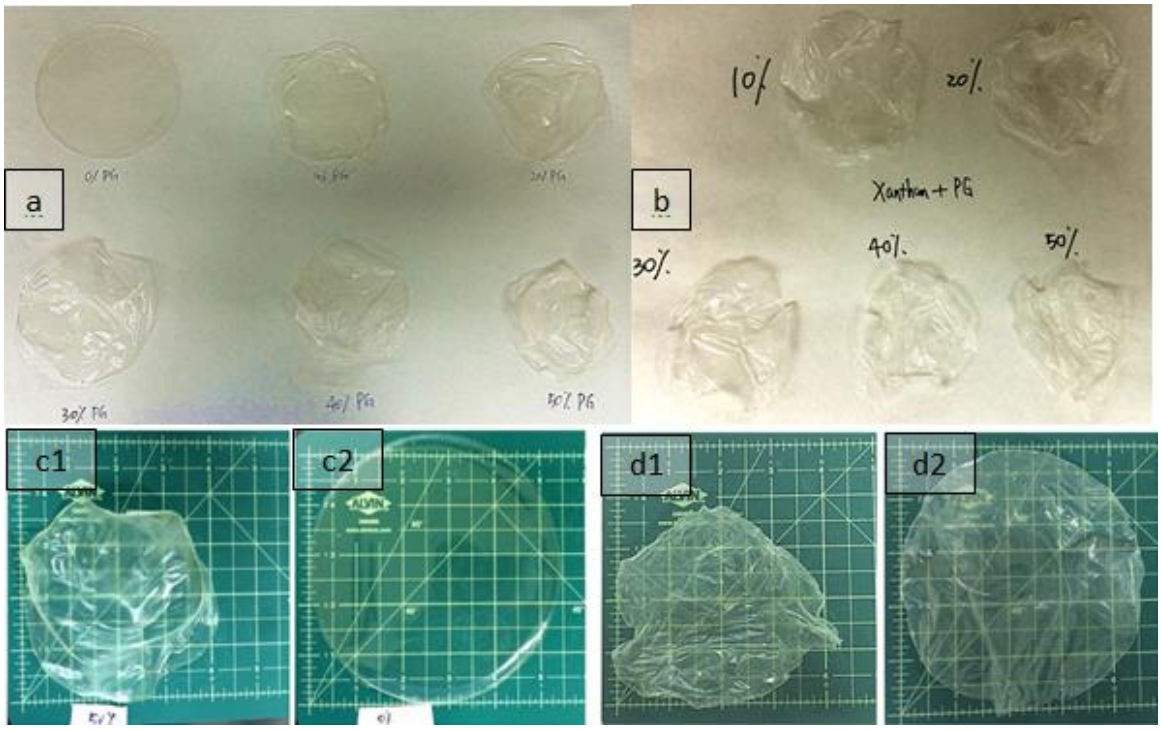

Figure 4.1 Films made from (a) 1% carrageenan+1% propylene glycol alginate with 0-50% propylene glycol (PG) and (b) 1% xanthan gum with 10-50% PG; and comparisons of films made from 1% carrageenan+1% propylene glycol alginate with 50% (c1) and 0% PG (c2), and 1% xanthan gum with 50% (d1) and 10% (d2) PG on scaled board.

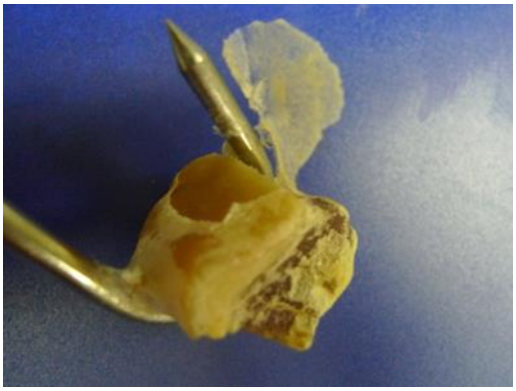

Figure 4.2 Dry-cured ham cube dipped in 50% propylene glycol and 1% carrageenan+1% propylene glycol alginate after 3 weeks storage at 24°C and 50%RH.

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