Meat Science 113 (2016) 73–79

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Developing food-grade coatings for dry-cured hams to protect against ham mite infestation

Y. Zhao [a], S. Abbar [b], T.W. Phillips [b], J.B. Williams [a], B.S. Smith [c], M.W. Schilling [a,*]

[a] Department of Food Science, Nutrition and Health Promotion, Mississippi State University, Mississippi State, MS, United States
[b] Department of Entomology, Kansas State University, Manhattan, KS, United States
[c] Hawkins, Inc., Birmingham, AL, United States

ARTICLE INFO

Article history:
Received 18 May 2015
Received in revised form 16 November 2015
Accepted 18 November 2015
Available online 19 November 2015

Keywords:
Dry-cured ham
Ham mite
Food grade coating

ABSTRACT

Dry-cured hams may become infested with ham mites, *Tyrophagus putrescentiae*, during the aging process. Methyl bromide is the only known available fumigant pesticide that is effective at controlling ham mite infestations in dry cured ham plants. However, methyl bromide will be phased out of all industries as early as 2015 due to its status as an ozone-depleting substance. Research was conducted to develop and evaluate the potential of using food-grade film coatings to control mite infestations, without affecting the aging process and sensory properties of the dry-cured hams. 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. Water vapor permeability was measured to estimate the impact of coatings during the aging process. It was evident that carrageenan/propylene glycol alginate coatings were permeable to moisture, which potentially makes them usable during aging.

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 very different based on the type of ham and the length of the aging process varies from 3 months 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. Female mites kept on wheat germ or yeast at 20 °C and 85% RH are able to lay up to 500 eggs during their life span. At 20 °C 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 temperatures usually ranges between 16 °C and 25 °C 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 United States (Rentfrow et al., 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 3240 kg has been approved for dry cured pork products in the United States in 2015(EPA, 2013). Exploring potential alternatives to control mite infestation 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 et al., 2002; Sánchez-Molinero, García-Regueiro, & Arnau, 2010; Sekhon et al., 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

* Corresponding author.
E-mail address: schilling@foodscience.msstate.edu (M.W. Schilling).

http://dx.doi.org/10.1016/j.meatsci.2015.11.014
0309-1740/

been rubbed with paste of lard on the surface prior to storage to prevent flies and bacteria for over 100 years (Smith, 1923). Abbar et al. (2013) recently reported that several legal food additives applied to the surfaces of small ham cubes would inhibit mite reproduction following forced inoculation with live mites. These results on mite inhibition with food-safe additives facilitated the research reported below.

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 breast and have shown inhibition against *Escherichia* 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; and 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 coatings that are developed are permeable to moisture; and 3) to determine if the use of coatings changes the sensory properties of the dry cured ham.

2. Materials and methods

2.1. Food-grade coating materials on ham cubes

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 was prepared in distilled water.

2.1.2. Ham preparation

Six dry-cured hams were purchased from a commercial ham plant. From each ham, seven 1.3 cm thick slices and five 2.5 cm thick slices were obtained. The 2.5 cm slices were then cut into 2.5 cm^3 cubes for the mite infestation study. Ham slices/cubes were dipped directly into mineral oil, propylene glycol, 10% potassium sorbate solution, and glycerin for 1 min and allowed to drip on a mesh colander for another min. Lard was applied directly by rubbing a thin layer to cover the entire area.

For sensory evaluation, five 1.3 cm thick slices from each ham were treated as described for the ham cubes with mineral oil, propylene glycol, potassium sorbate, glycerin and lard, respectively. Two additional slices from each ham were non-treated control slices. Slices were then vacuum-packed and stored at 4 °C 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, respectively. Another cube from ham was also randomly selected and freeze-dried until the water activity dropped to 0.65 on the surface and 0.8 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.

2.1.3. Mite infestation study

Twenty adult *T. putrescentiae* with 10 or more females per group were transferred onto each cube from a laboratory colony, and the cube was placed in a ventilated, mite-proof 130-ml glass canning jar for incubation at 25 ± 1 °C and 70% relative humidity. For the first mite infestation study with cubes dipped in pure or diluted solutions of test material, mites were incubated for 21 days to allow for reproduction. In subsequent testing, in which propylene glycol was formulated into coatings, the mites incubation time was 14 days. Resulting mite populations on ham cubes were counted at the end of the 3-week or 2-week incubation period using a dissecting stereo-microscope (Olympus Model SZX10, Olympus Surgical & Industrial America INC.). Only adult or immature mobile stages of mites were counted as representing the level of reproduction from the initial 20 mites at the beginning of the trial.

2.2. Development of film coatings with polysaccharide and propylene glycol

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. Polysaccharides were utilized for two reasons. First, if no carrier is used, the propylene glycol will evaporate and lose its effectiveness within 2–3 weeks. Using the coating also decreases the effective concentration of propylene glycol, thus reducing the cost of the treatment. Preliminary tests on polysaccharides suggested that both 50% and 98% 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), methylcellulose (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).

2.2.2. Solution preparation with polysaccharides and propylene glycol

To evaluate how different polysaccharides interact with propylene glycol, combinations were tested (Table 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, and solutions were stirred on magnetic stir plates until homogeneous. For hot water soluble polysaccharides, boiled water was used and the solutions were stirred on magnetic stir plates with heating elements until homogeneous. Metal meat hooks were used to dip ham cubes ($2.5 \times 2.5 \times 2.5$ cm^3) into the gel solutions for 10 s. Coated cubes were hung at 24 °C and 50% RH to determine the film-forming abilities of the tested combinations.

2.2.3. Ham preparation for mite bioassay

Three sets of dry-cured ham cubes ($2.5 \times 2.5 \times 2.5$ cm^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 trials, propylene glycol (Essential Depot, Sebring, FL) was combined with polysaccharide solutions (Table 2). Xanthan gum was solubilized at room temperature, and other polysaccharides were solubilized in boiling water on hot stirring plates and were 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 2). Three commercially aged hams were used during each trial. Two cubes from each ham were dipped in

Table 1
Combinations of different polysaccharides and propylene glycol (PG) at different ratios for coating tests (w/w).

Polysaccharide and %		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

PGA: propylene glycol alginate, ST: starch, CG: carrageenan, MC: methyl cellulose, XG: xanthan gum, SA: sodium alginate, n/a: not applicable.

Table 2
Polysaccharides and propylene glycol (PG) treatment combinations (w/w) and dipping temperatures for dry-cured ham cubes.

Polysaccharides	PG	Water	Dipping temp
First trial			
XG 1%	n/a	99%	Rm temp
Agar 2%	n/a	98%	40 °C
PGA 2%	n/a	98%	60 °C
PGA 1% + CG 1%	n/a	98%	60 °C
Control	n/a	100%	Rm temp
Second trial			
XG 1%	50%	49%	Rm temp
Agar 2%	50%	48%	30 °C
PGA 2%	50%	48%	60 °C
PGA 1% + CG 1%	50%	48%	60 °C
Control	n/a	100%	Rm temp
Third trial			
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%	28 °C
PGA 1% + CG 1%	10%	88%	30 °C
PGA 1% + CG 1%	20%	78%	35 °C
PGA 1% + CG 1%	30%	68%	40 °C
PGA 1% + CG 1%	50%	48%	60 °C

PGA: propylene glycol alginate, CG: carrageenan, XG: xanthan gum, n/a: not applicable.

each treatment 10 s, which led to 6 cubes (3 replications with 2 subsamples) per treatment in total.

2.3. Film characterization

2.3.1. Film preparation for thickness and water permeability measurements

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 2), with the exception of the pure xanthan gum solution since xanthan gum has very poor film forming capacity at the applied percentage. Solutions were poured into 150 × 15 mm plastic petri dishes to form a thin layer of films. To estimate the amount of the gel solution coated on ham per surface area, ham cubes with 2.5 cm 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 25 ± 0.1 g for all treatments. The films were dried out at 24 ± 0.5 °C and 50% ± 2% RH until the weight of films remained constant.

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.

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 though 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 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 25 °C. 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

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

where T is the film thickness (m). P is the saturation vapor pressure of water (Pa) at the 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 25 °C, the driving force was 3074 Pa.

2.3.4. Oxygen transmission rate

Oxygen transmission rate (OTR) of films made from propylene glycol alginate + carrageenan with 0%, 10% and 20% propylene glycol was measured using a Mocon OX-Tran® 2/21 (Mocon OX-TRAN® Model 2/21, Minneapolis, MN). The instrument complies with ASTM

F-1927 and uses a coulometric sensor to detect OTR through films. Tests were conducted with 100% oxygen, under 760 mm Hg at 25 °C and 50% RH. A mixture of nitrogen (98%) and hydrogen (2%) was used as the carrier gas. OTR was first measured on all film treatments using a Mocon OX-Tran® Model 1/50 (Mocon, Minneapolis, MN), but the coatings transmission properties did not remain constant enough throughout the testing to achieve appropriate, stable results. Based on this, a subset of samples was submitted to an outside source to determine if the films that would most likely be adapted to the industry were permeable to oxygen.

2.4. Sensory evaluation

Ham slices treated with lard, mineral oil, glycerin, propylene glycol, and potassium sorbate were evaluated. Coatings on ham slices were washed off with tap water at room temperature before cooking. 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 that were coded with 3-digit random numbers. Samples were presented to the trained panelists (6–8), each with greater than 30 h of experience in tasting dry cured ham 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. A negative control was applied to setup the baseline for the determination of difference. 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.

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 ham slices ($P < 0.05$). 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.

3. Results and discussion

3.1. Mite reproduction and sensory testing 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 3. Six cubes were freeze-dried to determine the effect of water activity on mite development. No differences existed ($P > 0.05$) among the control, freeze dried, and glycerin treatments, as all produced high numbers of mites. This indicates that glycerin ($P > 0.05$) does not inhibit mite reproduction. All of these treatments increased from 20 to greater than 200 mites after 3 weeks of incubation. 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 are ineffective at preventing mite infestation (Table 3). Lard and propylene glycol dipped ham cubes had the lowest mite numbers ($P < 0.05$) compared to either the control, freeze-dried or glycerin 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 preventing mite infestation at the benchtop level. Lard could likely be used to control mites once the product is done aging since it is not permeable to oxygen or moisture. However, it could not be used until after aging is complete, which limits its usability during commercial settings.

No differences ($P > 0.05$) were detected in sensory characteristics between control ham slices and slices treated with food grade ingredients (Table 3). 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; personal observations).

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 β-lactoglobulin 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 1. Since previous work showed that 50% propylene glycol mixed with water was effective at controlling mites (Abbar et al., 2013); polysaccharides from Table 1 were tested with concentrations ranging between 5 to 50% propylene glycol. The 5% level of PG was tested to minimize the cost of the coating. The 50% level of propylene glycol was selected since it was the minimum effective concentration at controlling ham mites when only a mixture of propylene glycol and water were used as a treatment to dip ham cubes. Adding 50% propylene glycol to the coating formulation affected the gel forming abilities of some of the tested polysaccharides. Starch, carrageenan, and sodium alginate (with added Ca^{2+}) formed good gels with pure water; however, with 50% propylene glycol, they either did not gel or formed a very weak

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

Treatment	Mite		Sensory	
	Mean	SEM	Mean	SEM
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

Means with same letter within each row are not significantly different ($P > 0.05$) using Tukey's Honestly Significant Difference Test.
Scale for sensory evaluation: 1-no difference, 2-slight difference, 3-moderate difference, 4-large difference, 5-very large difference.
n/a: not applicable.

Table 4
Mean number of mites on inoculated ham cubes (20 female mites/cube) coated with different polysaccharides and propylene glycol (PG) combinations after 2 weeks incubation.

Polysaccharides	PG	Mean	SEM
Control	0%	274^{a}	52.62
Agar (2%)	0%	111^{b}	18.82
PGA (2%)	0%	55^{bc}	7.91
XG (1%)	0%	29^{bc}	4.19
PGA (1%) + CG (1%)	0%	28^{bc}	5.47
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

PGA: propylene glycol alginate, CG: carrageenan, XG: xanthan gum.
Means with same letter within each row are not significantly different ($P > 0.05$) using Tukey's Honestly Significant Difference Test.

Table 5
Mean number of mites on ham cubes coated with polysaccharides and different percentage of propylene glycol (PG) after 2 weeks incubation.

		Mite	
Polysaccharides	PG	Mean	SEM
Control	0%	476[a]	48.72
Negative control	0%	n.a.	n.a.
PGA (1%) + CG (1%)	0%	186[b]	45.22
XG (1%)	0%	155[b]	54.05
XG (1%)	10%	70[bc]	48.24
PGA (1%) + CG (1%)	10%	2[c]	0.48
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

PGA: propylene glycol alginate, CG: carrageenan, XG: xanthan gum.
Means with same letter within each row are not significantly different ($P > 0.05$) using Tukey's Honestly Significant Difference Test.

gel. On the contrary, propylene glycol alginate formed a weak gel with pure water, but had very good gel forming capacity when combined with 50% propylene glycol. When propylene glycol alginate was combined with carrageenan, a very consistent film coating was formed on the ham surface with 50% propylene glycol. Xanthan gum formed a viscous and consistent gel with and without propylene glycol.

Based on results of the initial dipping tests in Table 1, selected polysaccharides were mixed with either 0 or 50% propylene glycol for initial mite mortality tests (first and second trials in Table 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 produced among these three coating treatments (Table 4). However, all coatings with 50% propylene glycol had absolutely no mites found alive after the 14 day incubation period (Table 4).

3.3. Coatings developed with polysaccharides and propylene glycol

Since the purpose of this research was to find an effective and economical alternative for methyl bromide, the cost of food-grade coatings should be formulated so that they are effective at minimal costs. Since xanthan gum and PGA + CG treatments numerically had the fewest mites in the previous experiment, these 2 treatments were selected for further testing to assess lower propylene glycol concentrations and thus reduce application costs. 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 5).

Similar to the results in Table 4, the 2 coating treatments with 0% propylene glycol were effective ($P < 0.05$) at reducing mite reproduction when compared to the control. When 10% PG was added to the PGA + CG treatment, the mite population was almost non-detectable levels with an average of 2 mites per jar ($P < 0.05$). This indicates that propylene glycol could potentially be added at concentrations as low as 10% and control mites on aging dry cured hams. All treatments with XG or PCA + CG with 20% PG or higher yielded no detectable mites after the incubation (Table 5). This indicates that XG was effective at controlling mites on ham cubes with concentrations of 20% PG and greater. These results infer that tests should be scaled up with whole hams in simulated aging houses

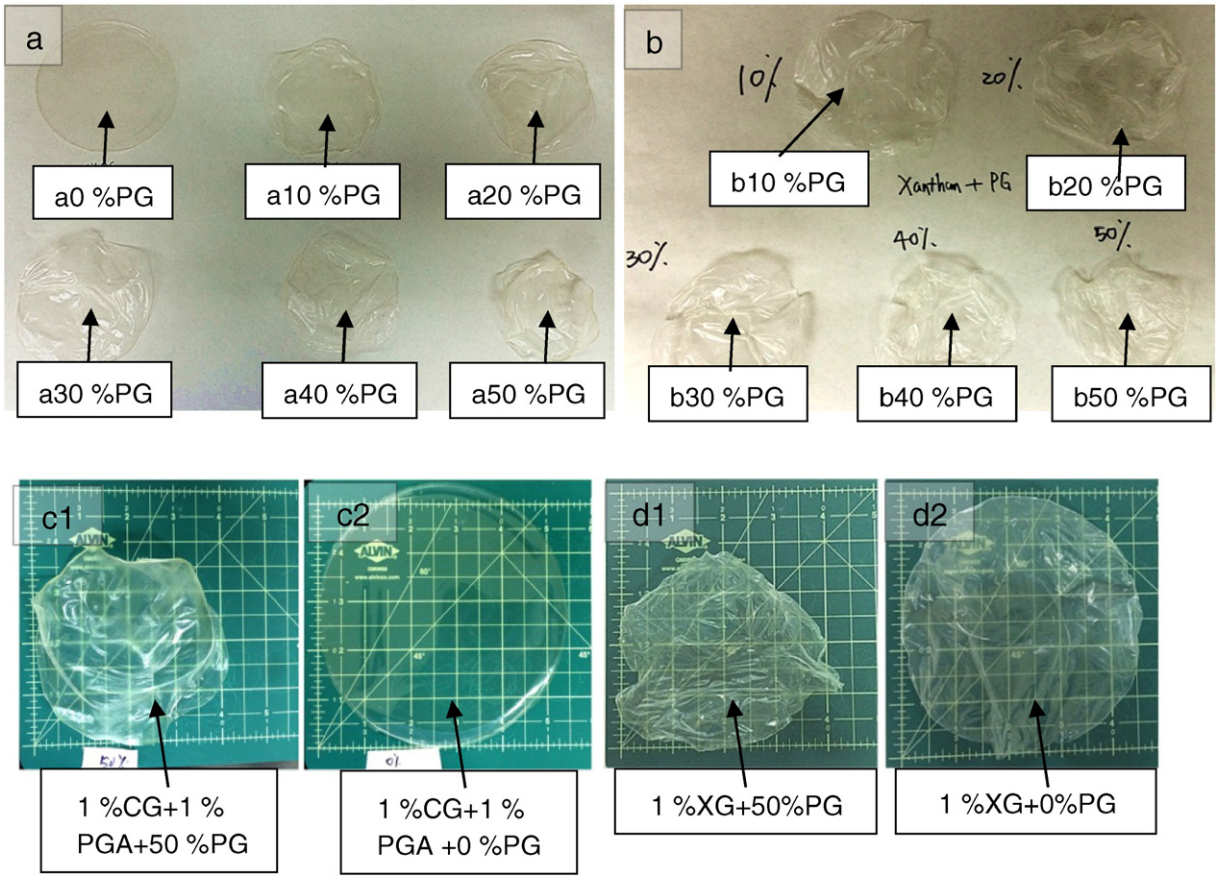

Fig. 1. Films made from (a) 1% carrageenan (CG) + 1% propylene glycol alginate (PGA) with 0% propylene glycol (PG) (a0), 10% PG (a10), 20% PG (a20), 30% PG (a30), 40% PG (a40), and 50% PG (a50) and (b) 1% xanthan gum (XG) with 10% PG (b10), 20% PG (b20), 30% PG (b30), 40% PG (b40), and 50% PG (b50); and comparisons of films made from c1) 1% CG + 1% PGA with 50% PG and c2) 1% CG + 1% PGA with 0% PG c2), and d1) 1% XG with 50% PG and d2) 1% XG with 10% PG on scale board.

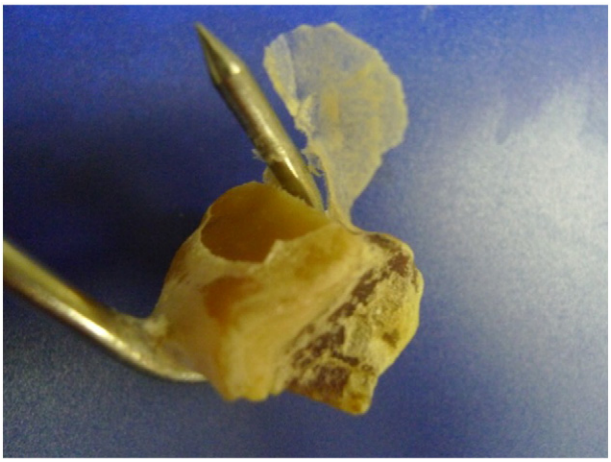

Fig. 2. Dry-cured ham cube dipped in 50% propylene glycol and 1% carrageenan + 1% propylene glycol alginate after 3 weeks under 24 °C and 50% RH.

and in commercial dry cured ham facilities with coatings similar to those tested here.

3.4. Film properties

3.4.1. Appearance

As a humectant, PG kept the films from drying out fast with 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 consistent weight at 25 °C and 50% RH. After dried out, films with PG all wrinkled to some extent (Fig. 1). Three possible explanations are: 1) different parts of the film dried at slightly different rates due to slight differences in PG distribution; 2) randomness of breaking/reforming of hydrogen bonds due to a slightly different surrounding environment; and 3) PG slightly affect 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 (Fig. 2).

3.4.2. Thickness

The thickness of films made from xanthan gum and propylene glycol alginate + carrageenan increased and demonstrated a linear and quadratic trend ($P < 0.05$) as propylene glycol percentage increased (Table 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 ($P < 0.05$) that 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 the decreased film areas due to shrinkage since increasing levels of PG lead to increases in shrinking during drying (Fig. 1).

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 6). There was a linear and quadratic increase ($P < 0.05$) in WVP for xanthan gum as propylene glycol concentration increased. Though not compared statistically, 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 highly permeable to water, which allows moisture loss, which is needed so that the dry cured ham is preserved during aging and meets the 18% moisture loss requirement by the United States Department of Agriculture (USDA, 1999). Propylene glycol is the functional ingredient in the film coatings for controlling mites. However, it could also be added as a plasticizer that has similar function as glycerol and sorbitol to reduce the brittleness of films. When used as plasticizer, the amount of propylene glycol added to the solution is usually between 10% to 60% by weight of the polysaccharide (Skurtys et al., 2010). Generally, 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 et al., 2010), similar to what was seen in the current research.

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 proper 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 for 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.

3.4.4. Oxygen transmission rate

Oxygen transmission rate (OTR) of films made from propylene glycol alginate + carrageenan with 0%, 10% and 20% propylene glycol was measured in duplicate. The average reading of films made

Table 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).

	1% XG				PGA 1% + CG 1%			
	Thickness (mm)		WVP (10^{-7} gPa^{-1} h^{-1} m^{-1})		Thickness (mm)		WVP (10^{-7} gPa^{-1} h^{-1} m^{-1})	
PG	Mean	Sem	Mean	Sem	Mean	Sem	Mean	Sem
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	0.03^{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%	0.02^{a}	0.00060	1.77^{a}	0.039	0.045^{a}	0.00076	3.47^{a}	0.107

Means with same letter within each row are not significantly different ($P > 0.05$) using Tukey's Honestly Significant Difference Test.
n/a: not applicable.

with 0%, 10% and 20% propylene glycol was 1.922 ml/(m^2·day), 1.953 ml/(m^2·day), and 1.876 ml/(m^2·day). These coatings show good barrier properties to oxygen and are similar in permeability to a lower molecular weight (%) EVOH resin (Mokwena, Tang, Dunne, Yang, & Chow, 2009). Since OTR of the films was relatively low, additional studies should be conducted to increase oxygen permeability, such as use of β-lactoglobulin (Sothornvit & Krochta, 2000), which forms an oxygen permeable film when combined with propylene glycol.

4. Conclusions

Coatings made from propylene glycol, xanthan gum and carrageenan + propylene glycol alginate are effective at preventing ham mite infestations on treated ham pieces under laboratory conditions. In addition, these coatings were permeable to moisture, which is essential to 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.

Acknowledgments

Approved for publication by the Mississippi Agricultural and Forestry Experiment Station under project MIS-325030, USDA-NIFA; also approved as publication no. 15-302-J by the Kansas Agricultural Experiment Station. This work was supported in part by the MAFES (MIS 35270 and 326050), the KAES and competitive grants received from USDA NRI Methyl Bromide Transitions Program (award number 2015-51102-2143).

References

Abbar, S., Zhao, Y., Schilling, M. W., & Phillips, T. W. (2013). Chemical alternatives for suppressing the ham mite Tyrophagus putrescentiae. *Paper presented at the Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions, MBAO conference. San Diego, CA* (pp. 36).

Aldrich, G. (2014). *Propylene glycol: When, where, and how should it be used.* Petfood Industry (Retrieved from http://www.petfoodindustry.com/articles/4304-propylene-glycol-when-where-and-how-should-it-be-used).

Alves, V. D., Costa, N., & Coelhoso, I. M. (2010). Barrier properties of biodegradable composite films based on kappa-carrageenan/pectin blends and mica flakes. *Carbohydrate Polymers*, *79*(2), 269–276.

Boczek, J. (1991). Mite pests in stored food. *Ecology and management of food-industry pest* (pp. 57–79). Arlington, Virginia: Association of Official Analytical Chemists.

EPA (2013). *Methyl bromide critical use renomination for post harvest dry-cured pork products.* United States (Retrieved from http://www.epa.gov/ozone/mbr/CUN2014/2014CUNPork%20.pdf).

Fields, P. G., & White, N. D. (2002). Alternatives to methyl bromide treatments for stored-product and quarantine insects. *Annual Review of Entomology*, *47*, 331–359.

García, N. (2004). Efforts to control mites on Iberian ham by physical methods. *Experimental & Applied Acarology*, *32*(1–2), 41–50.

Ghanbarzadeh, B., Almasi, H., & Entezami, A. A. (2011). Improving the barrier and mechanical properties of corn starch-based edible films: Effect of citric acid and carboxymethyl cellulose. *Industrial Crops and Products*, *33*(1), 229–235.

Macchioni, F., Cioni, P. L., Flamini, G., Morelli, I., Perrucci, S., Franceschi, A., & Ceccarini, L. (2002). Acaricidal activity of pine essential oils and their main components against *Tyrophagus putrescentiae*, a stored food mite. *Journal of Agricultural and Food Chemistry*, *50*(16), 4586–4588.

Mali, S., Grossmann, M. V. E., García, M. A., Martino, M. N., & Zaritzky, N. E. (2004). Barrier, mechanical and optical properties of plasticized yam starch films. *Carbohydrate Polymers*, *56*(2), 129–135.

Mokwena, K. K., Tang, J., Dunne, C. P., Yang, T. C., & Chow, E. (2009). Oxygen transmission of multilayer EVOH films after microwave sterilization. *Journal of Food Engineering*, *92*(3), 291–296.

Rao, M. S., Kanatt, S. R., Chawla, S. P., & Sharma, A. (2010). Chitosan and guar gum composite films: Preparation, physical, mechanical and antimicrobial properties. *Carbohydrate Polymers*, *82*(4), 1243–1247.

Rentfrow, G. R., Chaplin, R., & Suman, S. P. (2012). Technology of dry-cured ham production: Science enhancing art. *Animal Frontiers*, *2*(4), 26–31.

Rentfrow, G. R., Hanson, D. J., Schilling, M. W., & Mikel, W. B. (2008). The use of methyl bromide to control insects in country hams in the Southeastern United States. *Extension publication* (pp. 1–2). University of Kentucky Extension/National Country Ham Association (Publication# ASC-171).

Sánchez-Molinero, F., García-Regueiro, J. A., & Arnau, J. (2010). Processing of dry-cured ham in a reduced-oxygen atmosphere: Effects on physicochemical and microbiological parameters and mite growth. *Meat Science*, *84*(3), 400–408.

Sánchez-Ramos, I., & Castañera, P. (2000). Acaricidal activity of natural monoterpenes on *Tyrophagus putrescentiae* (Schrank), a mite of stored food. *Journal of Stored Products Research*, *37*(1), 93–101.

Sekhon, R. K., Schilling, M. W., Phillips, T. W., Aikins, M. J., Hasan, M. M., Corzo, A., & Mikel, W.B. (2010). Effects of phosphine and methyl bromide fumigation on the volatile flavor profile and sensory quality of dry cured ham. *Meat Science*, *86*(2), 411–417.

Seol, K. H., Lim, D. G., Jang, A., Jo, C., & Lee, M. (2009). Antimicrobial effect of κ-carrageenan-based edible film containing ovotransferrin in fresh chicken breast stored at 5 °C. *Meat Science*, *83*(3), 479–483.

Skurtys, O., Acevedo, C., Pedreschi, F., Enrione, J., Osorio, F., & Aguilera, J. M. (2010). *Food hydrocolloid edible films and coatings: Nova Science Publishers.*

Smith, H. H. (1923). Curing meat on the farm. *Colorado Agricultural College Extensive Service Bulletin* (211-A).

Sothornvit, R., & Krochta, J. M. (2000). Plasticizer effect on oxygen permeability of beta-lactoglobulin films. *Journal of Agricultural and Food Chemistry*, *48*(12), 6298–6302.

TEAP (2000). *Montreal protocol on substances that deplete the ozone layer: UNEP Technology and Economic Assessment Panel.*

Toldrá, F. (2010). Dry cured-ham. In F. Toldrá (Ed.), *Handbook of meat processing* (pp. 351–362). Chichester, UK: John Wiley & Sons.

USDA (1999). *"Country Ham", "Country Style Ham", "Dry Cured Ham", "Country Pork Shoulder", "Country Style Pork Shoulder", and "Dry Cured Pork Shoulder" (Meat and Poultry Inspection Regulations).* (Retrieved from http://www.gpo.gov/fdsys/pkg/CFR-2012-title9-vol2/pdf/CFR-2012-title9-vol2-sec319-106.pdf).

Ustunol, Z. (2009). Edible films and coatings for meat and poultry. In M.E. Embuscado, & K.C. Huber (Eds.), *Edible films and coatings for food applications* (pp. 245–268). New York: Springer.