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Application of hydrocolloid and propylene glycol infused nets and coatings on cave aged cheddar cheese and their impact on *Tyrophagus putrescentiae* growth and sensory properties

By

Kavitha Rama Krishnan

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

Mississippi State, Mississippi

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Application of hydrocolloid and propylene glycol infused nets and coatings on cave aged cheddar cheese and their impact on *Tyrophagus putrescentiae* growth and sensory properties

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The objective of this study was to evaluate the effects of using food grade coatings and nettings formulated with xanthan gum and propylene glycol (PG) or carrageenan (CG), propylene glycol alginate and PG on aged Cheddar cheese to control *Tyrophagus putrescentiae* growth at temperatures of 10°C, 15°C, and 20°C and relative humidity's of 75% and 85%. Cheddar cheese cubes with treated nets and coatings inhibited mite growth at all temperature and relative humidity combinations. Control cheese cubes either without coatings or in untreated nets had fewer mites ($P<0.05$) at 10°C than at 15°C or 20°C. The sensory properties of the cheese were not affected by the coatings and nettings at 10°C and 75 % RH. However, all other temperature and RH combinations with the exception of the CG netting at 15°C at 75 % and 85 % RH caused sensory flavor differences ($P<0.05$).

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DEDICATION

I would like to dedicate this thesis back to my Goddess Sarasvati and Lord Ganesha, who have given me the knowledge and strength to perform good work.

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I would first like to acknowledge and thank Dr. Wes Schilling for his guidance, dedication and support throughout my program of study. I am thankful to my committee members: Dr. Christine Cord, Dr. Courtney Crist and Dr. Thomas Phillips for their support throughout my research. I am grateful to all the members of the awesome lab: Xue, Yan, Jasmine, Morgan, Michael, Virell, Gisele, Doug, Nitin, Wenjie, and Soma for their support.

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CHAPTER I

INTRODUCTION

Cheddar cheese, first manufactured in Cheddar, England is one of the most commonly produced and consumed cheeses throughout the world, with 3.7 billion pounds produced in the United States in 2017 (USDA, 2018). Cheddar is a hard, ripened cheese without any surface flora that may range from white or ivory to light yellow or orange (Codex Alimentarius Commission, 2013; Walstra et al., 2006). Cave aged Cheddar cheese is bandaged and aged in caves, which provides nutty and rich flavor with distinct rinds. Caves are maintained at approximately 10-15°C and a relative humidity of 85 – 90%, which is an environment for mites to grow (Cheese Reporter, 2007). The finished cheddar cheese product should have a minimum of 50% milkfat, with a maximum moisture content of 39% (USDA FSIS 21 CFR 133.113). The taste of Cheddar cheese develops during ripening, which can take from three weeks to several years. A short ripening period produces a creamy taste and a longer ripening time produces a complex slightly nutty flavor (British Cheese Board - Cheddar, 2018).

Tyrophagus putrescentiae (Schrank), called the ham or cheese mite commonly infests dry stored foods such as grains, cured meats and aged cheese (Zhao et al.,2016a; Boczek, 1991). This species is attracted to pungent cheese flavors and high protein and fat content and thrives in areas where fungi can grow (Zhang et al., 2018). During aging, mites burrow into the cheese or enter through damaged coating while the cheese ages and

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leaves behind brown dust (mite powder) that consists of cast skins, excreta, debris, cheese crumbs and dead mites (Carvalho et al., 2018; Peace, 1983). In addition, cheese mite infestations result in allergic reactions for some humans and economic losses to the cheese industries (Carvalho et al., 2018). Hard and semi hard cheeses are most prone to mite infestation (Dawood and Ali, 2015).

Relative humidities of 65% or greater and temperatures ranging from 8.5°C – 36°C favor the growth of *T. putrescentiae* (Abbar et al., 2016; Aygun et al., 2007) and in general mold mites thrive at a relative humidity between 80 -90% (Aygun et al., 2007). Methyl bromide is a colorless, odorless fumigant that is effective at eliminating mites (Zhao et al., 2016a). Methyl bromide fumigation leaves no residue, is non-flammable and non-corrosive, and can easily diffuse in a given space to disinfect an entire area, such as a warehouse, grain bin or processing facility. However, methyl bromide use is no longer permitted for use by the cheese industry based on the Montreal Protocol (Fields and White, 2002). In response to the phase out of methyl bromide, several other chemical alternatives have been evaluated under laboratory conditions for their suitability to control mites. Sulfuryl fluoride and phosphine have been widely used to control mite infestations in nuts and grains. However, phosphine may not be applicable in industrial plants, due to the corrosion of copper wiring (Zhao et al., 2015). Under laboratory conditions, sulfuryl fluoride was ineffective against the *T. putrescentiae* egg stage at 3 times the legal label rate (Phillips et al., 2008).

Under laboratory conditions, results indicated that covering ham cubes in 100% lard or 50% - 100% of propylene glycol was effective at controlling mite reproduction over 14 d of incubation (Abbar et al., 2012). Based on these results from Abbar *et al.*,

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(2012), Zhao *et al*., (2016b) developed food grade coatings, 1% xanthan gum + 20 % propylene glycol or 1% propylene glycol alginate / carrageenan + 10% propylene glycol, that were effective at controlling *T. putrescentiae* under laboratory conditions. To ease the process of coating whole hams using these food grade ingredients, the ingredients were incorporated into nets. The nets incorporated with at least 40% propylene glycol controlled mite reproduction under laboratory conditions (Campbell et al., 2018).

The primary objective of this research was to evaluate the effects of using food grade coatings as dipping's directly on cave aged Cheddar cheese and in nets to control mite growth at temperatures of 10°C, 15°C and 20°C and a relative humidity of either 75 or 85%. An additional objective of this research is to evaluate the impact of food grade coatings and treated nets on the sensory perception of cave aged Cheddar cheese at the temperature and relative humidity combinations listed above.

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CHAPTER II

LITERATURE REVIEW

Cave aged cheddar cheese and manufacturing

Cheddar cheese has been manufactured in Cheddar, England since the middle ages and is one of the most consumed cheeses throughout the world (British Cheese Board - Cheddar, 2018). There are more than 500 varieties of cheeses produced in the world (International Dairy Federation, 1982). Two types of cheese are produced in the United States. Natural cheeses include unripened (Ex. cottage cheese), soft (Ex. camembert), semi-hard (Ex. roquefort) and hard cheeses (Ex. Cheddar). Processed cheeses are made by combining two or more different cheeses at some stage during the ripening process (EPA, 1995). Cheddar cheese was first produced in caves to store and ripen the cheese. Caves were used because they provide an optimum combination of relative humidity and temperature for the cheese flavor to mature (British Cheese Board - Cheddar, 2018). Caves are maintained at 10-15°C and a relative humidity of 85 – 90%, which is an ideal environment for mites to grow on bandaged cheese (Cheese Reporter, 2007). Cheddar cheese is classified as a hard cheese, with fat and moisture contents of approximately 50% and 38% respectively (Walstra et al., 2006). If unpasteurized ingredients are used, Cheddar cheese must be cured in an environment not less than 1.33 ^{o}C for at least 60 days. If pasteurized ingredients are used, phenol should not exceed 3 ug in 0.25 g of Cheddar cheese. The finished cheddar cheese product should have a

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minimum of 50% milkfat, with a maximum moisture content of 39% (USDA FSIS 21 CFR 133.113).

Cheddar cheese texture and flavor are dependent on salt content, water activity and pH. A salt content of 4% or less results in a soft consistency, whereas a salt content of 6% or greater makes the cheese hard and crumbly. Additionally, a pH of 5.6 or greater results in softer texture. Cheddar cheese has a pH of 5.2 to 5.3 and a salt content between 4 to 6% (Walstra et al., 2006). If the pH of the cheese decreases during processing, there is a spontaneous loss of colloidal calcium phosphate from the casein micelles, along with the dissociation and aggregation of sub-micelles, which results in changes in cheese texture, melting properties, and stretchability (Lawrence et al., 1987; Singh et al., 2003). Cheddar cheese consists of a correct balance of volatile flavor compounds which arise from the degradation of milk components by enzymatic activity, specifically citrate, casein, lactose and lipids. The flavor compounds that are produced vary in number and concentration as ripening time increases. An extensive list of volatile flavor compounds such as aldehydes, ketones, esters and sulfur containing compounds that contribute to Cheddar cheese flavor have been identified (Singh et al., 2003). Degradation pathways such as glycolysis, proteolysis and lipolysis, directly contribute to the formation of compounds that are responsible for cheese flavor, such as peptides, amino acids, short chain fatty acids, and diacetyl. This is followed by secondary catabolic changes that mainly take place during ripening such as decarboxylation, esterification, deamination, desulfurylation and beta oxidation of fatty acids. These changes contribute to cheese texture and flavor (Fox, 1993; Fox et al., 1994). Degradation of primary caseins such as alpha$_{S1}$- casein contribute to Cheddar cheese texture (Singh et al., 2003). Drake *et al*.,

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(2007) identified glutamic acid as a major contributor to umami flavor in Cheddar cheese (Drake et al., 2007).

Acidification and concentration involve the fermentation of lactose to lactic acid in Cheddar cheese manufacture, through the inoculation of *Lactococcus lactis* subsp. *cremoris* and subsp. *lactis* strains. Rennet causes the enzymatic hydrolysis of kappa casein, which leads to protein coagulation. Once the fresh cheese curd has formed, the process of cheddaring begins. The curd is sliced into small pieces to initiate faster syneresis (Walstra et al., 1987), and adequate acidity after which these pieces are subjected to sufficient salt, molded into their desired shape, pressed and ripened in curing rooms for a few weeks to as long as several years (Singh et al., 2003).

Infestations on cheese

The mites that most commonly infest aged cheese include *Tyrophagus putrescentiae* (Shrank) and *Acarus siro* (Hill, 2002; Hughes 1976; Yaman et al., 2000; Solomon, 1962). *Tyrophagus putrescentiae* (Schrank), commonly called "the cheese, mold or ham mite" is a ubiquitous species that commonly infests foods with high fat and protein contents (Aygun et al., 2007). Cheese mites are arachnids that are detected on the surface of cheese by visually identifying a "brown powder" that consists of dead and living mites, cuticles, debris and excreta (Carvalho et al., 2018). Mites are associated with economic losses to the cheese industry and certain health problems for plant workers such as diarrhea, enteritis, and systemic anaphylaxis (Armentia et al., 1994; Matsumoto, et al., 1996; Li et al., 2003). *Tyrophagus putrescentiae* has a translucent body that is barely visible to the naked eye (0.28-0.41 mm). They have a life span of 2-3 weeks under optimum environmental conditions, which are 25°C and a relative humidity of 65 % or

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greater (Hughes, 1961; Abbar et al., 2016). The brain and other organs of mites are in a compartment that is referred to as the idiosoma. The idiosoma is protected by hair-like setae that function as sensory structures (Chimileski, 2016). Female mites lay up to 500 eggs within their life cycle at a temperature of 20°C and 85% RH and complete one generation in 10-24 days. Once the eggs are developed, adult mites are full grown in approximately 9-12 days in a 20°C and 85% RH environment (Boczek, 1991).

Mite infestations are a common problem in dry stored products such as grains, meats, cheeses and hams. Aged cheeses are prone to mite infestations, especially during ripening since the environmental conditions are favorable for their growth (Carvalho et al., 2018). Aged cheese that is ripened in old boxes or storage cabinets are usually the primary area of infestation. Freshly made paraffins which are coated over the cheese are usually resistant to cheese mite infestations if the coating is not damaged. If the paraffin coating has been mishandled or if it has been cracked in certain areas, it provides an opening for mites to enter. Apart from damage to paraffins, contaminated environmental conditions in the curing room such as unclean shelves, walls, floors or dirty storage containers facilitate the growth and development of mites. Mites thrive at temperatures between 8.5°C to 36°C (Aygun et al., 2007) and relative humidities of 70 to 90% (Boczek, 1991). Some traditional cheeses such as the German Milbenkase and the French Mimolette are aged with stored mites to provide desired flavors and tastes (Hughes, 1959; Brückner and Heethoff, 2016; Melnyk et al., 2010).

Artisanal cheeses that are aged in giant caves can become infested on their rinds. *Tyrophagus species* feed on fungal hyphae such as the mold, *Scopulariopsis fusca* that grows on the cheese rinds. If vacuums, compressed air and/or brushes are not used when

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infestations have occurred, mites form a biofilm layer that is composed of fungi and bacteria that can cause mold spores from the rind to the rest of the cheese, which leads to spoilage (Chimileski, 2016).

Apart from *Tyrophagus putrescentiae,* other mite species that have infested aged cheeses include *Aleurobius farinae, Tyroglyphus siro* and *Tyroglyphus longior*. In the United States, different species of *Tyroglyphus* and *Tyrophagus* have been isolated from cheese (Carvalho et al., 2018). *Acarus siro* has infested Cheddar cheese in warehouses that are set at 85% RH and 12°C with the presence of the mold *Penicillium verrucosum* associated with increased mite reproduction and infestation (Peace, 1983). Dawood and Ali (2015) reported that the Egyptian artisanal cheese, Ras, can become infested by *A. siro*. Surk, a Turkish cheese that is consumed after 20 to 25 days of ripening at room temperature was reported to harbor *T. putrescentiae* (Guler, 2000).

Methods to control mite infestations

Chemical and physical methods have been implemented to control mites in the dairy industry. Methyl bromide was used until the Montreal Protocol was signed in 1992 as a means to control mites on aged Cheddar cheese (Brunner et al., 2000). Specialized vacuum cleaners are used in the cave aged Cheddar cheese industry to remove loose mite dust on the surface of cheeses, along with compressed air and brushes to blow mites off the rinds (Chimileski, 2016). Regular cleaning of curing rooms, scrubbing of cheese boxes, careful handling of cheeses, changing the paraffins of the cheese and maintenance of RH at 43% or less can decrease mite activity (Cheese Reporter, 2013). A study on the effect of different essential oils such as clove, rosemary, thyme and citrus revealed that

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clove oil was the most effective at controlling *A. siro*, (Dawood and Ali, 2015). However, the use of these oils imparted undesirable flavor to the cheese.

Methyl bromide

Methyl bromide is a colorless, odorless fumigant that has been used in multiple industries to control a variety of pests, including cheese mites. It is currently used to control ham mites in the dry cured ham industry in the United States. In studies conducted by The Department of Agriculture, Ontario Agricultural College, two fumigants were effective at controlling cheese mites. The first compound was a combination of 6.8 % methyl bromide with 93.2% carbon dioxide and the second was 10% ethylene-oxide with 90% carbon dioxide (Price, 1938). However, methyl bromide was listed as an ozone layer depleting substance in 1987 in the Montreal Protocol (Fields and White, 2002; Brunner et al., 2000). In this agreement, 182 countries signed an agreement put forth by the United Nations to phase out the use of ozone depleting substances (UNEP, 1992). Methyl bromide was phased out of use by all industries on January 1st, 2005, with the exception of critical use exemptions or pre-shipment and quarantine uses (EPA, 2017).

Commercial methyl bromide fumigations have been carried out in warehouses, coolers, factories and curing rooms, which do not contain an outlet for the gas to escape. In dairy plants, methyl bromide has predominantly been used to control pests such as roaches, mites and cheese skippers, which may be found during aging or storage. Methyl bromide is effective at eradicating all arachnid forms including eggs. Some of the main reasons why methyl bromide is a desirable fumigant is its potency across a wide range of temperatures, ease of application in an industrial setting, immiscibility in water and lack

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of effect on the sensory quality of treated products (Punoo, 2016). Dry cured ham industries have used methyl bromide to help control mite infestations. However, as of 2017, the annual applications of MBTOC for critical use exemptions were stopped, and dry cured ham processors are currently allowed to use methyl bromide stockpiles until they are depleted (EPA, 2017). Due to limitations to methyl bromide use, and decreasing supplies, economically viable alternatives are necessary to prevent mite infestations across a wide range of industries (Zhao et al., 2016a). This has led to efforts by researchers across academics and food industries to investigate potential replacements for methyl bromide.

Alternatives to methyl bromide

Chemical alternatives to methyl bromide include carbon dioxide, phosphine, sulfuryl fluoride and ozone, which have been studied under laboratory conditions for their effectiveness at controlling *Necrobia rufipes* (red legged beetles) and *T. putrescentiae* (Sekhon et al., 2010a, b, c). Phosphine was effective at controlling mites on dry cured ham under laboratory and scaled-up conditions (Zhao et al., 2015). Dry cured hams were subjected to different concentrations of phosphine (First trial - 1000 ppm for 48 hr, 2800 ppm for 48 hr; second trial – 2000 ppm for 48 hr and 2500 ppm for 48 hr; third trial - 2000 ppm for 48 hr and 2500 ppm for 72 hr). Twenty mites were inoculated in ventilated glass jars that contained ham slices and maintained at 28°C with 60% RH. Under laboratory conditions, there was 100 % mortality of all developmental stages of ham mites at 1000 ppm phosphine in a 48-hour period, when counted 3 weeks post incubation. However, phosphine gas was highly corrosive to copper fittings in an industrial trial, even though it was effective at controlling mites (Phillips et al., 2012;

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Sekhon et al., 2010a; Zhao et al., 2015). Therefore, phosphine fumigation is not an acceptable method to control mites in most dry cured ham or aged cheese plants, but it could potentially be used to disinfest hams and other materials that are in isolated fumigation chambers or other structures for which corrosion is a concern.

Sulfuryl fluoride (SF) was registered in 2005 to control pests in the dry cured pork industry (EPA, 2005). However, fumigation had to be conducted at SF concentrations that were greater than the permissible fumigation rate (1,500 g-hrs per cubic meter) that is approved by the EPA to achieve high mite mortality of *T. putrescentiae.* When applied at room temperature for a 48 hr period on a mixture (n = 50) of adult *T. putrescentiae* and *N. rufipes*, a concentration that was 3 times greater than the legal limit of about 100.3g/m^3 led to 95 % mortality of the egg stage of *T. putrescentiae* mites and 100% mortality of adult mites (Phillips et al, 2008). Hence, using SF at such high concentrations is not feasible.

Carbon dioxide (CO_2) can be used to control pests that affect grains, cheeses and dry cured hams since it decreases aerobic metabolism, which is needed to support life. Twenty *T. putrescentiae* were incubated with small amounts of food at 23°C at 65% to 85% RH. To control mites, 62.5% CO_2 exposure for 144 h was required for 100% mortality of eggs and mobile stages of the cheese mite, *T. putrescentiae* at 20°C (Hasan et al., 2016). As a result, use of high levels of carbon dioxide is impractical in ham and cheese industries due to the need to shut down plant operations for an extended period of time, which would lead to economic losses. Ozone may help control *T. putrescentiae* on surfaces. However, due to its lack of ability to penetrate organic material, it would not be

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a viable alternative for aged cheese since mites can enter into cracked paraffin and burrow in to the cheese (Hasan et al., 2016).

Food grade ingredient coatings and nettings

Food-grade ingredient based coatings have been developed to deliver active ingredients that prevent and control mite infestations on dry-cured hams. Cooking oils have been used, on plants to prevent mite and insect infestations through suffocation (Hoy, 2011, Lewis, 2014). Abbar *et al*., (2016) dipped 1 cm^3 cubes of hams into food grade ingredients including propionic acid, butylated phenol derivatives, short-chain alcohols, citric acid, and animal and vegetable oils prior to inoculation with 20 mites and incubation for 14 d at 25°C and 70% RH (Abbar et al., 2016). Results indicated that 100% lard, and 50 % 1, 2 propanediol (propylene glycol, PG), and 100% PG controlled mite growth. Based on the results of Abbar *et al*., (2016), food grade coatings were developed by Zhao *et al.* (2016) that consisted of carrageenan, propylene glycol alginate, xanthan gum, and PG, since lard is impermeable to oxygen and moisture. Dry-cured ham cubes (15.7 cm^3) were dipped into these coatings, allowed to dry and then inoculated with 20 mites prior to incubation at 24°C – 26°C and 70% RH. It was confirmed that using 1% xanthan gum + 20 % propylene glycol and 1% propylene glycol alginate / carrageenan + 10% propylene glycol was effective at controlling mite reproduction over 2 to 3 weeks of incubation time under laboratory conditions (Zhao *et al.*, 2016). Zhao *et al.*, (2016) reported that propylene glycol at concentrations between 20 -50% had no more than two mites present on each ham cube sample. As the concentrations of PG increased, the rate of oxygen transmission decreased and water vapor permeability increased (Zhao et al., 2016). Since nets and hooks are commonly used to hang hams to racks, the food grade

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coatings were incorporated into nets and evaluated for their efficacy at controlling ham mites. Nets that were infused with coatings with at a minimum of 40% PG controlled mite reproduction under laboratory conditions (Campbell et al., 2018).

Zhang *et al.* (2017) treated ham nets that were used to hang dry cured hams during aging with the xanthan gum and carrageenan/propylene glycol alginate solutions that contained PG. These treated nets were placed on ham cubes (15.7 cm^3) that were divided into three groups. Each of these groups (mites inoculated on day 1, mites inoculated 4 weeks after placing ham cubes in the nets, and 8 weeks after placing ham cubes in the nets) were inoculated with 20 mites and maintained at temperatures of 21°C to 25°C. After a 2 week period, only four mites were present on treated cubes in nets that contained medium and high concentrations of PG in comparison to untreated cubes, which harbored more than 200 to 300 mites (Zhang et al., 2017). In addition, Hendrix *et al*., (2018) used nets that were infused with carrageenan, propylene glycol alginate, xanthan gum and propylene glycol to control ham mites on both ham slices (2.5cm x 9 cm x 15.5cm) and cubes (15.7 cm^3) at relative humidity and temperature combinations of 55%, 65%, 75% and 85% and 24°C, 28°C and 32°C respectively (Hendrix et al., 2018). Coated nets were effective at controlling mites at all temperature and relative humidity combinations evaluated. When nets were not coated, mite growth was greatest at 24ºC and 65 % RH, 28ºC and 65 % RH, 28ºC and 75 % RH, and 32ºC and 75 % RH. Behavioral studies indicated that mites have a tendency to orient themselves towards untreated net (controls) over treated ham cubes (Zhang et al., 2017; Abbar et al., 2016).

The following set of criteria have been reported by different researchers to choose food grade coatings for use on cheese. 1) coatings must decrease oxygen permeability

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since oxygen facilitates lipid oxidation and growth of pathogenic organisms, 2) coatings must facilitate an increase in shelf life, 3) decrease water loss and 4) decrease exposure of the cheese to light since light also facilitates lipid oxidation (Lin and Zhao, 2007; Kester and Fennema 1986; Nisperos-Carriedo, 1994). Since food grade ingredient coatings have been effective at controlling *T. putrescentiae* in ham, this technology may be effective at controlling mite infestations on aged Cheddar cheese.

Relative humidity and temperature

The temperature and relative humidity of cave aged cheese curing impacts flavor development, development of surface flora and rinds, and prevention of spoilage due to mold. Cheese caves are man-made storage areas in which cheeses are aged at a particular temperature and relative humidity combination that is dependent on the desired flavor profile (Rotronic Measurement Solutions, 2014). Prior to the 1900's, the temperature was maintained in cheese curing rooms at 23°C (Miah et al., 1974). Currently, cooler temperatures are maintained, depending on the kind of cheese that is aged. Semi-hard and hard cheeses, such as cave aged Cheddar cheese require temperatures of approximately 10°C and relative humidity of approximately 85-90% (Cheese Reporter, 2007). During the manufacture of Cheddar cheese and many related cheese varieties, temperatures of 31°C–39°C are maintained. Many Cheddar-type cheeses are then aged in storage rooms or caves at 15.6°C or slightly below (Bishop and Smukowski, 2006).

Peace (1983) indicated that *Acarus siro* increased in number on Cheddar cheese at 11°C–13°C and 85–88% RH (Peace, 1983). *Tyrophagus putrescentiae* reproduces between temperatures of 10.4°C and 34.8°C, with greater mite reproduction growth as the temperatures increases (Sánchez-Ramos and Castañera, 2005). Hendrix *et al.,* (2018)

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used different combinations of food grade ingredient treated coatings infused in nets on ham cubes and subjected them to different temperature and relative humidity of 24°C, 28°C, and 32°C and 55, 65, 75, and 85% RH respectively and reported that the 85% RH with all temperature combinations slowed mite reproduction. However, the water activity, (A_w) of the ham also impacts mite reproduction and growth and should also be considered.

The relative humidity and temperature in the cheese caves affects the water activity and moisture content of cheeses. Generally, the moisture content of Cheddar cheese is 39% or less (Bishop and Smukowski, 2006), and the water activity is approximately 0.95 (Bishop and Smukowski, 2006) and varies as the cheese ages. In semi hard cheeses, water activity is the most important factor that determines cheese stability, which is dependent on the salt and moisture content (Cerqueira et al., 2009). Maintenance of relative humidity contributes to the overall quality of dry cured hams. Dry cured hams are aged at RH of 65% to 85%, and temperatures of 28°C or greater, which are conditions that support mold growth and mite reproduction (Sánchez-Molinero, García-Regueiro, & Arnau, 2010). However, with respect to cave aged Cheddar cheese, research has not been reported on the effects of temperature and relative humidity on mite growth and sensory quality.

Sensory effect of food grade coatings

Cerqueira *et al.,* (2009) studied the effects of edible functional polysaccharides including chitosan, agar from *Glacilaria birdiae* and galactomannan from *Gleditsia triacanthos* with different combinations including corn oil and plasticizers on a regional Saloio cheese. The coatings were effective at controlling surface mold that commonly

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grows on cheese (Cerquiera et al., 2009). Rambod *et al.,* (2018) conducted a study on the effects of using xanthan gum and flaxseed mucilage in food grade coatings on the shelf-life, microbial stability, and chemical stability of Cheddar cheese during their ripening process. Sensory analysis was performed on a blind label basis and consumers evaluated the sensory attributes on a hedonic scale from 1 (extremely dislike) to 5 (strong positive). Their results indicated that the coatings caused changes in free fatty acid content, pH, and titratable acidity. However, the sensory qualities such as cutting, flavor, texture, and color were not affected by the coatings. Flavors were improved by xanthan gum, 0.75% flaxseed mucilage and 1% flaxseed mucilage when compared to the noncoated cheese. Xanthan gum had the highest scores compared to flaxseed mucilage for color, cutting, texture and flavor. Use of 1.25% flaxseed mucilage had a slight negative effect on the color score of Cheddar cheese (Rambod et al., 2018). Studies on the effect of sensory quality after the application of food grade coatings that have been previously used to control mites on dry-cured ham have not been conducted on aged Cheddar cheese. However, in whole ham studies by Campbell et al., (2018), cotton nets were infused with carrageenan, propylene glycol alginate, xanthan gum and propylene glycol. These nets were placed on whole hams at dry cured ham facilities, and did not negatively impact flavor, moistness and texture (Campbell et al., 2018). Therefore, it would be worthwhile to evaluate food grade coatings on Cheddar cheese for their efficacy at controlling mite reproduction and to understand their effect on sensory quality.

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CHAPTER III

MATERIALS AND METHODS

Preparation of coatings and nets infused with food grade coatings

Carrageenan (CG) (Ticagel® 795 Powder, TIC Gums), propylene glycol (PG) (Essential Depot, Sebring, FL), propylene glycol alginate (PGA) (Tica- algin®, PGA, TIC Gums) and xanthan gum (XG) (Pre-hydrated Ticaxan Rapid-3 powder, TIC Gums, Belcamp, MD 21017) were used in the preparation of food-grade coatings and net infusions. Two different types of coatings were formulated. The first coating was made from a combination of 1% CG + 1% PGA + 40% PG, and the second coating was formulated with 1% XG and 40% PG. For the first coating type, CG and PGA were mixed with PG with constant stirring. While stirring, water was added and heated until the liquid became clear (90-100˚C) since CG and PGA are hot water-soluble gums. For the second coating type, XG, a cold-water polysaccharide, was dissolved in tap water at 20ºC (room temperature) prior to the addition of PG.

A netting machine (Midwest Metalcraft & Equipment Company, MI 65360) was used to infuse coatings into a 50% polyester and 50% cotton blend netting (Ennio Meat Packaging Specialists, Smoke Nets, Aurora, IL, 60504) within one hour of preparation of each coating solution. The nets were cut, weighed and dipped in the coatings. The coated nets were then pressed between the two rollers on the netting machine. The amount of

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coating that was absorbed by the nets were measured and reported as g/m. The 1% CG, 1% PGA and 40% PG nets absorbed 190 ± 5 g/m and the 1% XG and 40% PG nets absorbed 183 ± 5 g/m. The nets were then vacuum packaged with a dual-chamber ULTRAVAC vacuum packaging machine (Model UV2100, Koch Equipment, Kansas City, MO) in bags (3 mil. standard barrier, nylon/PE Clarity Vacuum Pouches; Kansas City, MO) and stored at 20°C until they were applied as treatments.

Cheese preparation

Three separate blocks of Farmhouse Cheddar cheese (Jasper Hill Farm, Greensboro Bend, VT) were selected from 3 different lots of Cheddar cheese that were aged for approximately 9 months. Cheeses were cut (Handee cheese cutter, Cheese and Yogurt Making, NJ) into 5 cm x 5 cm x 5 cm cubes for sensory evaluation and 2.5 cm x 2.5 cm x 2.5 cm cubes for mite reproduction assays. Cubes were then dipped in coatings for 1 min by tying thread around the cheese cubes and dipping them in solutions or placing them in treated and untreated nets (control). Control samples were not dipped in the coating.

Mite cultures

Prior to use, *Tyrophagus putrescentiae* were shipped to Mississippi State University from the department of entomology at Kansas State University. The mites were transferred into the diet mixture that was prepared as explained by Abbar et al., (2016). Mites were reared biweekly at Mississippi State University in ventilated 355 mL glass jars (Wide-mouth jar, Ball Mason, Ball Corp., Broomfield, CO) that contained fresh mite diet (Abbar. *et al*., 2016). The ventilated jars were covered with filter paper (Whatman No. 1, 90 mm diameter; GE Healthcare UK Limited, Amersham, UK) and

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sealed with the jar ring. The jar was placed in a locked storage box that contained soapy water on the bottom, and petroleum jelly smeared on the edges of the container to prevent mites from escaping. The mite culture was maintained at 25 °C and 75 - 85 % RH in a dark cabinet for three to four weeks prior to use.

Mite infestation assays

The 2.5cm x 2.5cm x 2.5cm cubes were randomly assigned in triplicate to six different treatments: control (untreated cubes), CG+PGA+PG coatings, XG+PG coatings, untreated control nets, CG+PGA+PG nets and XG+PG netted treatments. Ventilated glass canning jars (216 mL, 65 mm diameter, 55 mm height; Ball Corp., Broomfield, CO) with black construction paper (14 cm diameter) at the bottom were used to conduct the assays. Each cheese cube was placed in a jar and inoculated with twenty mixed-sex, adult *Tyrophagus putrescentiae*. The jars were then covered with filter paper for ventilation, placed in a container filled with soapy water which covered one third of the bottom of the jars, and surrounded with petroleum jelly to prevent mites from escaping. Triplicate jars that contained the untreated cubes, CG+PGA+PG coated cubes, XG+PG coated cubes, untreated control netted cubes, CG+PGA+PG netted cubes and XG+PG netted cubes were placed together in a 0.28 cubic meter pesticide treated environmental control chamber (LH-10 Economy Line Humidity Chamber, Associated Environmental Systems, Acton, MA). Samples were exposed to temperatures of 10˚C, 15˚C or 20˚C for two-week periods with a relative humidity of either 75% or 85%. Each temperature was tested in combination with each specified relative humidity.

Post incubation, the cheese cubes were removed from the jars along with the filter paper, black construction paper and netting and placed in separate Petri dishes. A

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microscopic evaluation (Model 568, American Optical Company, Buffalo, NY) of each component was carried out to count the number of living mites on the filter paper, black construction paper and netting material (Hendrix et al., 2018).

Water activity and moisture content

Water activity and moisture content were measured for each Cheddar cheese sample before and after 14 d of incubation in the environmental chamber. Prior to inoculation, cheese cubes were randomly selected to measure the water activity (AquaLab Series 3 TE, Decagon Devices, Inc., Pullman, WA). The water activity and moisture content of all the untreated and treated cheese cubes (n=30) were measured after storing the cubes for at least 48 hrs in the freezer to ensure all mites have been killed, prior to measurement. Moisture content of the cheese samples were determined by mincing the cheese cubes. 2.0 ± 0.1 g of minced cheese was dried in the oven at 100°C for 3-4 h until a steady weight was obtained (AOAC, 1998). Once the dried weight was obtained, the moisture content was obtained using the following formula:

$$\%\ Moisture = \frac{Initial\ Cheese\ Weight(g) - Dried\ Cheese\ Weight(g)}{Dried\ Cheese\ Weight\ (g)} * 100$$

Sensory evaluations

Difference from control tests were performed on cheese samples to determine if experienced panelists (n=6-8, >20 hours serving on descriptive analysis panels) could detect a difference between control Cheddar cheese samples and treated Cheese samples. Cheese blocks (125 cm^3) were randomly assigned to and then exposed to one of the following six treatments: control (untreated cubes), CG+PGA+PG coatings, XG+PG coatings, untreated control nets, CG+PGA+PG nets and XG+PG nets. Treatments were

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then placed in ventilated glass jars (216 mL, 65 mm diameter, 55 mm height; Ball Corp., Broomfield, CO) and incubated for two weeks at temperatures and relative humidity combinations of 10˚C, 15˚C or 20˚C and 75% or 85% RH. After the incubation period, the nets were removed from the cheese cubes and coatings were retained on the cubes at 4˚C until sensory evaluation occurred. Each block of Cheddar cheese was cut into eight cubes that were approximately 1 x 1 x 1 cm in dimension. The cheese cubes were prepared one hour prior to testing and placed in 29.5 ml clear sample cups (RS6BPY, 125/5 7/8-inch, Sweetheart Cup Company Inc. Chicago, IL). Random 3-digit numbers were labeled on the sample cups and a difference from control test was conducted. A labeled control sample was given to set the baseline to determine the difference between treated and untreated cheese. A blind control was included in the assignment of treatments to anchor the differences between the treatments and the control.

Panelists were selected from the university based on availability, previous training, and knowledge of Cheddar cheese. Experienced panelists (n=6-8, with 3 panels per trial, which was 36 panels, 3 replications x 6 RH and temperature combinations x 2 treatments (coating/nets)) were asked to taste the control sample first, and then and evaluate all other samples in a randomized order to account for sampling bias. Panelists were asked to evaluate differences with respect to color, aroma, flavor, texture, and moistness. Panelists were seated in separate booths for each panel and were provided with unsalted crackers (Premium, Nabisco, NJ), water (Mountain Spring Water, Blue Ridge, GA), apple juice, napkins, forks and expectoration cups. To cleanse their palate during a 20 second break between samples, water, apple juice and unsalted crackers were provided. The scale that was used for difference from control test was: 0 = no difference,

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1 = slight difference, 2 = moderate difference, 3 = large difference, 4 = very large difference (Civille and Carr, 2015).

Statistical analysis

A 3 (10°C,15°C,20°C) x 2 (75 %, 85 %) factorial arrangement within a completely randomized design with three replications was used to evaluate the effect of temperature and relative humidity on *T. putrescentiae* population growth, water activity and moisture content of cheese cubes for each of the six separate treatments: control (untreated cubes), CG+PGA+PG coatings, XG+PG coatings, untreated control nets, CG+PGA+PG nets and XG+PG netted treatments. For difference from control tests, a randomized complete block design with three replications, was used to determine if panelists (n= 6 to 8, with 36 panels that consisted of 3 replications x 6 RH and temperature combinations x 2 treatments (coating/nets)) detected differences with respect to color, aroma, flavor, texture and moisture between treated and untreated aged Cheddar cheese cubes ($P < 0.05$). SAS statistical software (SAS 9.4, 2013, SAS Inc., Cary, NC) was used to conduct statistical analysis. When significant differences occurred among treatments, Tukey's Honestly Significance Difference Test ($P < 0.05$) was used to separate treatment means ($P<0.05$).

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CHAPTER IV

RESULTS AND DISCUSSION

Mite reproduction assays

Untreated cheese cubes - coating controls

No interaction existed ($P>0.05$) between temperature and relative humidity with respect to their impact on mite counts. When averaged over relative humidity (RH), mite counts were fewer ($P<0.05$) on cheese cubes at 10°C when compared to 15°C and 20°C. No differences existed in mite counts ($P>0.05$) between the 15°C and 20°C treatments (Table 4.1). When averaged over temperature, there was no difference ($P>0.05$) in mite reproduction at each RH evaluated (Table 4.2). When means were separated for temperature and relative humidity combinations, the 10°C treatment at 75% RH had fewer counts ($P<0.05$) than the 15°C and 20°C treatments at 75% RH. In addition, the 10°C treatment at 85% RH also had fewer counts ($P<0.05$) than the 15°C and 20°C treatments at 85% RH. However, the 10°C treatment at 85 % RH did not differ in counts from the 15°C and 75% RH treatment, which shows a slight trend for greater counts at the higher relative humidity treatment with mean mite counts ranging from 21.8 to 90.0 (Table 4.3).

Carrageenan + propylene glycol alginate + propylene glycol coatings

The CG + PGA + PG coatings controlled mite reproduction better than untreated cubes, as evidenced by harboring less than the initial inoculation level of 20 mites.

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Interaction existed ($P<0.05$) between temperature and relative humidity with respect to their impact on number of ham mites. When averaged over RH, mite counts were fewer ($P<0.05$) on cheese cubes at 15°C when compared to 10°C, but no differences existed ($P>0.05$) between 10°C and 20°C and 15°C and 20°C with respect to mite counts (Table 4.1). When averaged over temperature, mite counts were fewer ($P<0.05$) at 85% RH when compared to 75% RH (Table 4.2). When means were separated for temperature and relative humidity combinations, the 10°C and 15°C treatments had fewer ($P<0.05$) mites at 85% RH than 75% RH, but no differences existed ($P>0.05$) in mite counts between 75% and 85% RH at 20°C. Regardless of temperature and RH combination, the carrageenan coating was effective at controlling mites since mean counts ranged from 1.7 to 7.5, which were all less than the initial inoculation level of 20 mites (Table 4.3).

Xanthan gum + propylene glycol coatings

Interaction existed ($P<0.05$) between temperature and relative humidity with respect to mite counts. When averaged over RH, mite counts were fewer ($P<0.05$) on cheese cubes at 15°C and 20°C than at 10°C, and mite counts did not differ ($P>0.05$) between the 15°C and 20°C treatments (Table 4.1). When averaged over temperature, mite counts were fewer ($P<0.05$) at 85% RH than 75% RH (Table 4.2). These results are similar to those for the CG treatments. Mite counts were fewer ($P<0.05$) at 85% RH than 75% RH at both 10°C and 20°C, but did not differ ($P<0.05$) among RH at 15°C. In addition, regardless of temperature and RH combination, the xanthan gum coating was effective at controlling mites since mean counts ranged from 1.4 to 7.8, which were all less than the initial inoculum level of 20 mites (Table 4.3).

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Untreated cheese cubes – nets

The mean mite counts ranged from 10.4 to 70.0 (Table 4.6) with no interaction ($P>0.05$) between temperature and relative humidity with respect to their impact on mite counts. When averaged over relative humidity (RH), mite counts were fewer ($P<0.05$) on cheese cubes at 10°C when compared to 15°C and 20°C (Table 4.4). In addition, there were fewer mites than the 20 mites from the initial inoculation level for the 10°C treatment, which indicates that mites were controlled at 10°C but not at 15°C or 20°C, when only nets were used (Table 4.5). When means were separated for temperature and relative humidity combinations, the 10°C treatment at 75% RH and 85 % RH also had fewer counts ($P<0.05$) than all other temperature and RH treatments (Table 4.6). At each temperature that was evaluated, there was no difference ($P>0.05$) in mite counts between the 75% and 85% RH treatments. Results indicated that keeping the temperature cold at 10°C had a greater impact on mite control than adjusting RH.

Carrageenan + propylene glycol alginate + propylene glycol nettings

Interaction did not exist ($P>0.05$) between temperature and relative humidity treatments with respect to mite counts. When averaged over relative humidity (RH), mite counts were fewer ($P<0.05$) on cheese cubes at 20°C than at 10°C, but no differences existed ($P>0.05$) between the 15°C and 20°C treatments (Table 4.4). When averaged over temperature, there were fewer mites ($P<0.05$) at 85% than 75% RH (Table 4.5). For each temperature treatment, there were fewer mites ($P<0.05$) at 85% RH than 75% RH. Results were very similar to the CG coating treatments. The carrageenan net treatments were effective at controlling mites since mean counts ranged from 3.0 to 8.7, which are all less than the initial inoculum level of 20 mites (Table 4.6).

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Xanthan gum + propylene glycol nettings

For mite counts, there was no interaction (P>0.05) between temperature and RH. When averaged over RH, mite counts were fewer (P<0.05) on cheese cubes at 15°C and 20°C when compared to 10°C. Mite counts did not differ (P>0.05) between 15°C and 20°C treatments (Table 4.4). These treatments are similar to xanthan gum coating treatments. When averaged over temperature, mite counts were fewer (P<0.05) at 85% RH than at 75% RH (Table 4.5). When means were separated for temperature and RH combinations, the 75% RH treatments had fewer counts (P<0.05) for both the 10°C and 15°C treatments, but no differences existed (P>0.05) in mite counts between the 75% and 85% RH at 20°C.The xanthan gum net treatments were effective at controlling mites since mean counts ranged from 2.0 to 8.1, which were all less than the initial inoculum level of 20 mites (Table 4.6).

The Cheddar cheese cubes that were untreated or in control nets had *T. putrescentiae* mite counts that were much greater than Cheddar cheese cubes in food grade coatings or coating treated nets that were formulated with CG + PGA+ 40% PG and/or XG + 40% PG. In addition, Cheddar cheese cubes that were stored at 10°C and 75% or 85 % RH had fewer mites than the control cubes exposed to 15 or 20°C at either relative humidity. This indicates that maintaining aging rooms at 10°C or less may be a control measure that could be used to slow mite growth and control infestations. Although minimal research has been conducted on using coatings on cheese to control pest infestations, various studies have supported the use of these coatings to prevent mite growth on dry cured hams, another aged product. Regardless of the temperature or the relative humidity, all XG + 40% PG and CG + PGA + 40% PG effectively inhibited the

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growth of *T. putrescentiae* when compared to the untreated cubes. These results are similar to Zhao *et.al*, (2016), who incorporated different food grade coatings on 2.5x2.5x2.5 cm^3 ham cubes and inoculated 20 adult mites on the ham cubes prior to incubation at 24 – 26°C and 70% RH for 14 and 21 d. Results indicated that XG + 20% PG and CG + PGA + 10% PG or higher concentrations of PG inhibited *T. putrescentiae* reproduction having 0 and 2 mites respectively (Zhao et al., 2016). Results were also similar to Zhang *et al*., (2017), where XG and CG+PGA coatings with medium PG had fewer mites on ham cubes then the initial inoculum of 20 mites after the ham cubes had been encased in nets for either 4 or 8 weeks prior to inoculation and incubated for 2 weeks (Zhang et al., 2017). Campbell *et al*., (2017) determined the minimal PG concentration that was necessary to inhibit *T. putrescentiae* growth on dry cured ham cubes. The cubes (2.5 cm x 2.5 cm x 2.5 cm) that were coated with either XG + 15% PG or CG + PGA + 7.5% PG were the lowest concentrations that were effective at inhibiting mite growth under laboratory conditions for each coating over 2 a week incubation time (Campbell et al., 2017). Zhang *et al*., (2018) incorporated nets with lard, PG, XG and other polysaccharides onto 2.5cm^3 ham cubes, and found that the mite count significantly reduced with treatments containing PG when compared to untreated cubes, which increased mite count from 20 mites to an average of 123-163 after a 10 week storage period (Zhang et al., 2018). Hendrix *et al*., (2018) studied the effects of temperature, RH and nettings on mite infestations of dry cured ham slices (2.5 cm x 9.0 cm x 15.5 cm) and 2.5cm^3 cubes. Untreated nets had a lower mite count compared to the initial inoculum level of 50 mites at 85% RH with 24°C, 28°C and 32°C combinations. Ham cubes and slices also showed that at 85% RH, nets infused with XG + PG or CG + PGA + PG

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completely inhibited mite growth of *T. putrescentiae*, in addition to CG + PGA + PG treatment reducing mite reproduction at all temperatures (24°C, 28°C and 32°C) and RH (55%, 65%, 75%, and 85%) respectively (Hendrix et al., 2018).

Apart from using these food grade ingredients to prevent *T. putrescentiae* growth, research has also been conducted on using natural and chemical methods to prevent mite infestation. Hasan et al., (2016) studied the efficacy of using controlled atmospheric and determined that low oxygen levels and high carbon dioxide levels of up to 144 hr of exposure time to cause approximately 100% mortality of *T. putrescentiae.* Twenty *T. putrescentiae* were used with small amounts of food and were incubated at 23°C at 65% to 85% RH. Though use of CO_2 was effective under laboratory conditions, it would not be feasible for use in dry cured ham plants since it would cause a plant to be shut down for approximately 144 h or 6 d, and it would be difficult to seal a plant to maintain concentrations (Hasan et al., 2016). Under laboratory conditions, 175 ppm of ozone was applied for 48 hrs, reporting a 100% mortality rate of *T. putrescentiae*, however due to the lack of ozone's ability to penetrate through crevices on ham surfaces, it reduces its killing effect as mites burrow themselves in cracks and crevices (Sekhon et al., 2010c; Hasan et al., 2016). Although work has been done to develop various methods on dry cured hams to prevent mite infestations, few researchers also worked on methods to reduce mite growth on different cheese types. Dawood and Ali (2015) evaluated the essential oils of thyme, rosemary, clove and citrus for their ability to inhibit *Acarus siro* infestations on ras cheese during ripening period. Corn oil was used to dilute the essential oils into their respective concentrations before usage. Slices of Ras cheese (4 cm x 4cm) were wiped with different concentrations of the essential oils and inoculated with 20

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mites (Dawood and Ali, 2015). The cheese slices were stored in the dark at 16°C-18°C and observed after 0, 30, 60 and 90 days under laboratory conditions. Use of 0.1 % clove oil led to 95% mite mortality. Use of 0.1 % rosemary (0.1 %) or thyme oil (0.1 %) led to 55% and 75% mortality respectively. However, all of these oils negatively impacted the flavor of the cheese. Overall, the oils had a greater effect on the cheese flavor as storage time increased (Dawood and Ali, 2015). Ramos and Castanera, (2009) reduced the ripening temperature of cabrales cheese from 15°C to 2,4 and 6°C, which reduced the number of *Acarus farris* mites from 141-207 mites to 1, 11 and 14 mites per cm^2. Their most efficient chemical method was nonanoic acid, which led to 63.4 % mite mortality as compared to controls with 26.1% mortality (Sánchez-Ramos and Castañera, 2005). The results of this current study indicated that regardless of the relative humidity and temperature combination, CG + PGA+ 40% PG and XG + 40% PG coating and netted treatments maintained a mite count of below the initial inoculum of 20 mites, indicating greater mite control than was seen in untreated cubes.

Water activity and moisture content

Untreated cheese cubes - coating controls

The average water activity (A_w) and moisture content (MC) ranged from 0.879 to 0.911 and 20.2 to 32.1, respectively (Table 4.3). No interaction ($P>0.05$) existed between temperature and relative humidity for A_w but existed ($P<0.05$) for MC. When averaged over RH, there was no difference ($P>0.05$) in A_w at each temperature evaluated, but the MC was less ($P<0.05$) at 10°C and 15°C than 20°C (Table 4.1). When averaged over temperature as expected, the water activity was lower ($P<0.05$) at 75% RH compared to 85% RH but no differences existed in MC (Table 4.2). When means were separated for

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temperature and relative humidity combinations, the 20°C treatment at 75% RH had a lower A_w ($P<0.05$) than the 20°C treatment at 85% RH, but no other differences existed between RH at both the 10°C and 15°C treatments. The 20°C treatment at 75% RH had a greater MC than the 15°C treatment at 75% RH. No differences existed ($P>0.05$) in MC between all treatments at 85% RH. However, at 75% RH, Cheddar cheese cubes that were exposed to 20ºC had greater moisture ($P<0.05$) than Cheddar cheese cubes at both the 10ºC and 15ºC treatments, and the 10ºC treatment cubes had more moisture ($P<0.05$) than the 15ºC treatment (Table 4.3).

Carrageenan + propylene glycol alginate + propylene glycol coatings

The average A_w and MC ranged from 0.882 to 0.914 and 25.0 to 33.3 respectively (Table 4.3). No interaction existed between temperature and relative humidity with respect to A_w but existed ($P>0.05$) with respect to MC. When averaged over RH, there were no differences ($P>0.05$) in A_w among the temperatures that were evaluated, but the MC was less ($P<0.05$) at 10°C and 15°C than 20°C (Table 4.1). When averaged over temperature, there was no difference ($P>0.05$) in A_w and MC between RH (Table 4.2). When means were separated for temperature and relative humidity combinations, there were no differences in A_w between 75% and 85% RH at 10°C, 15°C, or 20°C. For MC, the 20°C treatment at 75% RH had a greater MC than 10°C treatment at 85% RH and 15°C treatment at 75% RH. No differences existed ($P>0.05$) between treatments at 10°C (Table 4.3).

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Xanthan gum + propylene glycol coatings

The average A_w and MC ranged from 0.899 to 0.917 and 26.4 to 33.5 respectively (Table 4.3). No interaction existed ($P>0.05$) between temperature and relative humidity with respect to A_w, but there was interaction ($P<0.05$) present for MC. When averaged over RH, there was no difference ($P>0.05$) in A_w but the MC was less ($P<0.05$) at 10°C and 15°C than 20°C at each temperature evaluated (Table 4.1). When averaged over temperature, there was no difference ($P>0.05$) in A_w. However, the 75% RH treatment had less MC ($P<0.05$) than the 85% RH. (Table 4.2). When means were separated for temperature and relative humidity combinations, none of the treatments differed from each other ($P>0.05$) with respect to A_w. The MC was greater for Cheddar cheese cubes that were exposed to at 20°C and 85% RH than at 15°C and 75% RH. All 75% RH treatments differed ($P<0.05$) from each other with the highest moisture content at 20ºC and the least MC at 15ºC (Table 4.3).

Untreated cheese cubes – nets

The average A_w and MC ranged from 0.879 to 0.911 and 21.0 to 33.0, respectively (Table 4.6). Significant interactions existed ($P<0.05$) between temperature and RH for both A_w and MC. When averaged over RH, there was no difference ($P>0.05$) in A_w but the MC was less ($P<0.05$) at 10°C and 15°C than 20°C (Table 4.4). When averaged over temperature, the A_w and MC were less ($P<0.05$) at 75% RH than 85% RH (Table 4.5). When means were separated for temperature and relative humidity combinations, the 15°C treatment had a lower ($P<0.05$) A_w at 75% RH than at 85% RH, and no other differences existed ($P>0.05$) among RH within each temperature treatment. For MC, the 15°C treatment at 75% RH had less ($P<0.05$) MC than the 20°C treatment at

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75% RH. All temperature treatments at 85% RH did not differ (P>0.05) in MC (Table 4.6).

Carrageenan + propylene glycol alginate + propylene glycol nettings

The average A_w and MC ranged from 0.894 to 0.913 and 20.4 to 30 for carrageenan, propylene glycol alginate, and propylene glycol coated nets (Table 4.6). Interaction was present (P<0.05) between temperature and RH for both A_w and MC. When averaged over RH, there were no differences (P>0.05) between temperature treatments with respect to A_w, but the MC was less (P<0.05) at 10°C and 15°C than 20°C (Table 4.4). When averaged over temperature, there was no difference (P>0.05) in A_w. However, the 75% RH treatment has less (P<0.05) MC than 85% RH at each temperature that was evaluated (Table 4.5). When means were separated for temperature and RH combinations, the 10°C treatment at 85% RH had less (P<0.05) A_w than the 10°C treatment at 75% RH. No other differences (P>0.05) existed in A_w between RH treatments for both the 15°C and 20°C treatments. For MC, the 15°C treatment at 75% RH had less (P<0.05) MC than the 20°C treatment at 75% RH (Table 4.6). All temperature treatments at 85% RH did not differ (P>0.05) from each other with respect to MC.

Xanthan gum + propylene glycol nettings

The average A_w and MC ranged from 0.897 to 0.911 and 24.0 to 31.2 respectively (Table 4.6). No interaction existed (P>0.05) between temperature and RH for A_w however existed (P<0.05) between temperature and RH with respect to MC. When averaged over RH, there was no difference (P>0.05) in A_w with respect to temperature, but the MC was

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less ($P<0.05$) at 15°C than at 20°C (Table 4.4). When averaged over temperature, there was also no difference ($P>0.05$) in A_w between the RH treatments. However, the 75% RH treatment had less ($P<0.05$) MC than the 85% RH treatment (Table 4.5). When means were separated for temperature and RH combinations, there were no differences ($P>0.05$) in A_w between RH treatments at each temperature that was evaluated. The 15°C treatment at 75% RH had less ($P<0.05$) moisture content than the 20°C treatment at 75% RH (Table 4.6). In addition, no differences existed ($P>0.05$) between all treatments at 85% RH.

Storage of cheese cubes at 10, 15, and 20°C did not affect water activity. All A_w's were between 0.84 and 0.93, which was similar to the initial A_w of the cheese prior to the application of treatments and storage, which was between 0.88 to 0.94. Storage of cheese cubes at 75% RH and 85% RH impacted the water activity of only control cubes and control nets, indicating that the treatments did not influence the water activity. All MC's were between 20.2 to 33.5, which was slightly lower than the initial MC before treatments were applied which were between 32.0-36.7 with an initial pH of 5.1. The A_w values are different than those reported by Hendrix *et al.,* (2018), where increasing temperature affected the water activity of ham cubes and slices. Ham slices with similar coating treatments were maintained at 55, 65, 75, and 85% RH with temperatures of 24, 28 and 32°C. XG + PG net treated hams had decreased water activity and moisture content as the temperature increased. This may have been due to a larger spread in A_w since there were no differences in water activity between ham samples that were stored at 75 and 85 % RH and the moisture content only differed between 75 and 85 % RH at 32 C for CG + PGA + PG treatments.

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Sensory evaluations: difference from control test

10°C 75% RH coating

Cheese cubes treated with CG + PGA+ 40% PG and XG + 40% PG were not different ($P>0.05$) from the blind control cheese cubes with respect to color, aroma, flavor, texture and moistness (Table 4.7). Despite a lack of statistical differences, 20% of panelists commented that the treated cubes were more bitter and dry than the control cheese.

15°C 75% RH coating

Cheese cubes treated with CG + PGA+ 40% PG and XG + 40% PG were not different ($P>0.05$) from the blind control cheese cubes with respect to color and aroma (Table 4.7). There were differences ($P<0.05$) with respect to flavor, texture and moistness of the cheese cubes treated with CG + PGA+ 40% PG and XG + 40% PG in comparison to the blind control cheese. The mean ratings for flavor were 1.9 and 2.2 (moderate difference) for the XG and CG treatments, in comparison to 0.8 (slight difference) for the blind control. Panelists (20 %) indicated that the CG and XG treated cubes were more bitter and astringent than the controls. The mean ratings for texture and moistness were both approximately 1.0 and 1.1 for the XG and CG treatments, in comparison to 0.4 for the blind control, which indicates only a slight difference from the control.

20°C 75% RH coating

Cheese cubes treated with CG + PGA+ 40% PG and XG + 40% PG were not different ($P>0.05$) from the blind control cheese cubes with respect to color and aroma (Table 4.7). There were differences ($P<0.05$) with respect to flavor, texture and moistness

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of the cheese cubes treated with CG + PGA+ 40% PG in comparison to the blind control cheese. The mean ratings for flavor were 1 and 1.5 (slight to moderate difference) for the XG and CG treatments, in comparison to 0.3 (no to slight difference) for the blind control. However, the XG treatment and blind control were statistically similar. Panelists (20%) indicated that XG treatments had a nutty flavor compared to the control cubes. The mean ratings for texture and moisture were between 0.3 to 0.8 and 0.2 to 1.1, indicating between no difference and a slight difference.

10°C 85% RH coating

Cheese cubes treated with CG + PGA+ 40% PG and XG + 40% PG were not different ($P>0.05$) from the blind control cheese cubes with respect to color, aroma, texture and moistness (Table 4.8). Flavor differences did exist ($P<0.05$) between cheese cubes treated with CG + PGA+ 40% PG and XG + 40% PG in comparison to the blind control cheese. The mean flavor ratings were 1.7 and 2.0 for CG and XG treatments, which indicates a moderate difference from the control in comparison to the blind control, which was slightly different from the control with respect to flavor. Thirty percent of panelists indicated that the XG treated cubes had a slightly oxidized taste, and 20% of the panelists commented that both the XG and CG treated cheese cubes were more bitter and had a sharper taste than the control cubes.

15°C 85% RH coating

Cheddar cheese cubes were different ($P<0.05$) with respect to color and aroma for CG + PGA+ 40% PG coated treatment in comparison to the blind control (Table 4.8). However, the XG + 40% PG treatment was not different ($P>0.05$) from the blind control

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or CG + PGA+ 40% PG treatment. The CG + PGA+ 40% PG treatment had color and aroma ratings of 1.5 and 1.3 in comparison to 0.7 and 0.6 for the blind control, indicating a slight difference in color and aroma. The flavor ratings for both the CG and XG treatments indicated that their flavor was different ($P<0.05$) from the blind control. The mean ratings for flavor were 1.8 and 2.2 for the XG and CG treatments which indicates a slight to moderate difference from the control, in comparison to 1.1 for the blind control, which was indicative of slightly different from the control. This may be due to 30% of panelists indicating that the treated cheese cubes had a strong bitter taste in comparison to the blind control. Cheese cubes treated with CG + PGA+ 40% PG and XG + 40% PG were not different ($P>0.05$) from the blind control cheese cubes with respect to texture and moistness.

20°C 85% RH coating

Cheese cubes treated with CG + PGA+ 40% PG and XG + 40% PG were not different ($P>0.05$) from the blind control cheese cubes with respect to color, aroma, texture, and moistness (Table 4.8). The flavor of the Cheddar cheese cubes that were coated with CG + PGA+ 40% PG and XG + 40% PG were different ($P<0.05$) from the blind control cheese. The mean ratings the CG and XG treatments flavor were 2.1 and 2.4, which indicates that these cheese samples were moderately different from the control with respect to flavor. These differences may have been due to 20% of panelists indicating that the treated cheese cubes lacked cheese flavor, had a strong bitter off taste, and a soft texture when compared to the control cubes.

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10°C 75% RH nets

Cheese cubes treated with CG + PGA+ 40% PG and XG + 40% PG were not different (P>0.05) from the blind control cheese cubes with respect to color, aroma, texture moistness, and flavor (Table 4.9). Despite the lack of statistical difference in flavor, 20% of the panelists commented that the treated cheese cubes were more bitter than the control cubes.

15°C 75% RH nets

Cheese cubes treated with CG + PGA+ 40% PG and XG + 40% PG were not different (P>0.05) from the blind control cheese cubes with respect to color, aroma, texture and moistness (Table 4.9). There were differences (P<0.05) with respect to flavor of the cheese cubes treated with CG + PGA+ 40% PG and XG + 40% PG in comparison to the blind control cheese. Even though there were differences between the control cheese cubes and treated cubes with respect to flavor, the highest mean rating was 1.7, indicating a slight to moderate difference. Panelists (20%) commented that CG + PGA+ 40% PG PG treated cubes had a softer texture than the blind controls.

20°C 75% RH nets

Cheese cubes treated with CG + PGA+ 40% PG and XG + 40% PG were not different (P>0.05) from the blind control cheese cubes with respect to color and aroma (Table 4.9). Flavor, texture and moistness attributes were not conducted due to the presence of excess mold, which made the cheese inedible.

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10°C 85% RH nets

Cheese cubes treated with CG + PGA+ 40% PG and XG + 40% PG were not different (P>0.05) from the blind control cheese cubes with respect to color, aroma, texture and moistness (Table 4.10). However, there were no differences (P<0.05) with respect to the flavor of the cheese cubes treated with CG + PGA+ 40% PG in comparison to the blind control cheese. However, the XG + 40 % PG treatment had slightly different flavor (P<0.05) than the control. The mean ratings for flavor were 1.3 and 1.5 for the treated cubes (slight to moderate difference), however no difference (P>0.05) was seen between the blind controls and CG + PGA+ 40% PG treatments. Panelists (20%) indicated that the XG + 40% PG treated cubes were very bitter and different compared to the blind control cubes.

15°C 85% RH nets

Cheese cubes treated with CG + PGA+ 40% PG were not different (P>0.05) from the blind control cheese cubes with respect to color, flavor, aroma, texture and moistness (Table 4.10). XG + 40% PG treated cubes were not used for any panels due to excess mold growth.

20°C 85% RH nets

Cheese cubes treated with CG + PGA+ 40% PG and XG + 40% PG were not different (P>0.05) from the blind control cheese cubes with respect to color and aroma (Table 4.10). Flavor, texture and moistness attributes were not conducted due to the presence of excess mold, making the cheese inedible.

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All coating treatments compared to netted treatments caused flavor differences, except for 10°C and 75% RH, which did not impact the sensory properties of the cheese. This indicates that these coatings could only be used in the industry in the aging of Cheddar cheese if the temperature and relative humidity were controlled at or below these temperatures and relative humidity. Similar results were obtained with the application of netting with the exceptions of the 10°C treatment at 75% RH and the 15°C treatments at 75% or 85% RH. This indicates that even though the coatings and nets imparted a bitter taste to some of the cheese samples, the netting provided some protection against sensory differences, which is evidenced by fewer statistical differences and smaller difference ratings when compared to the coated cheese samples. Although research has been conducted on the incorporation of food grade coatings on cave aged Cheddar cheese with respect to different relative humidity and temperature combinations, other researchers have incorporated coatings onto different foods. Zhao et al., (2016b) treated dry-cured hams slices with lard, mineral oil, PG, glycerin and 10% potassium sorbate. PG and lard were effective at controlling *Tyrophagus putrscentiae* on ham cubes and did not impact the sensory properties of dry cured ham slices that were dipped in these compounds (Zhao et al., 2016b). This may have been because coatings were washed off and hams were cooked prior to sensory testing. Additional research on dry cured ham indicated that there were no differences with respect to the flavor, texture and moistness with ratings having a slight to moderate difference from the control samples for PGA + CG + 20% PG treatments while XG + 20% PG treatments and blind control were rated slightly different from the control samples after slicing whole hams into 1.3 cm thick slices after aging the hams with food grade coating infused nets for 4 months.

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This may be due to using less coating, rather than dipping method to infuse coatings, which affect the sensory quality of hams (Campbell et al., 2018). Campbell et al., (2017) indicated that there were some differences in flavor, texture and moistness when whole hams were dipped into food grade coatings when compared to the control samples. Slight to moderate differences in flavor were seen with hams treated with PGA + CG + 40% PG net treatment, PGA + CG only, and XG + 20% PG when compared to the blind control ham samples, along with slight and moderate differences seen in PGA + CG + 40% PG treatment with moistness and texture attributes respectively. However, the results were variable due to aging factors, processing plants, ham size and origins. Dipping whole hams into food grade coatings caused sensory differences that were not seen with spray coating, since spray coating left a thinner, uniform coating on hams (Campbell et al., 2017). Rambod *et.al,* (2018) incorporated 0.5% XG +50% glycerol and 0.75% flaxseed mucilage +50% glycerol on Cheddar cheese to be used as edible coatings. Results indicated that 0.75% XG and 1% flaxseed mucilage treatments did not negatively impact the flavor, texture, color and cuttability of Cheddar cheese, and enhanced the flavor of the cheese when compared to control samples (Rambod et al., 2018). These results are contrasting to results obtained from the XG + 40% PG treatments on cave aged Cheddar cheese, which imparted a bitter flavor in comparison to the control samples. The findings in dry cured ham studies were more promising than those for aged Cheddar cheese, with small differences that either did not impact overall quality or was not different at all. Thus, in order to adapt this technology of applying food grade solutions as dipping's or nettings to Cheddar cheese, the aging room temperature and relative humidity would need to be minimized to 10°C and 75 % RH or less and there would need to be

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technology employed to remove the coating or net prior to packaging and retail sales, to reduce the effect of the sensory quality on the cheese.

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Table 4.1 Mean values for mite infestation, water activity, and moisture content of cave aged Cheddar cheese cubes (2.54 cm x 2.54 cm x 2.54 cm) for untreated cubes, carrageenan, propylene glycol alginate, and propylene glycol coated cubes, and xanthan gum and propylene glycol coated cubes at different temperatures, when averaged over relative humidity after 14 d of incubation

Treatment	Temperature (°C)	Mite count On cheese cubes (No.)	Water activity	Moisture Content (%)
Untreated cubes	10	28.7[b]	0.889[a]	26.0[b]
	15	77.2[a]	0.903[a]	25.4[b]
	20	84.4[a]	0.889[a]	30.5[a]
	SEM	8.5	0.005	1.2
CG + PGA+ 40% PG	10	5.1[a]	0.893[a]	27.0[b]
	15	3.1[b]	0.897[a]	28.0[b]
	20	3.5[ab]	0.907[a]	32.0[a]
	SEM	0.5	0.007	0.9
XG + 40% PG	10	5.5[a]	0.907[a]	28.0[b]
	15	2.2[b]	0.913[a]	26.4[b]
	20	2.5[b]	0.900[a]	32.2[a]
	SEM	0.46	0.005	1.0

a-b: means with the same letter by column are not different (P>0.05) due to temperature within each coating control, coating with carrageenan, and coating with xanthan gum.
SEM: standard error of the mean

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Table 4.2 Mean values for mite infestation, water activity, and moisture content of cave aged Cheddar cheese cubes (2.54 cm x 2.54 cm x 2.54 cm) for untreated cubes, carrageenan, propylene glycol alginate, and propylene glycol coated cubes, and xanthan gum and propylene glycol coated cubes at different relative humidities, when averaged over temperature after 14 d of incubation

Treatment	Relative humidity (%)	Mite count On cheese cubes (No.)	Water activity	Moisture Content (%)
Untreated cubes	75	55.5[a]	0.885[b]	26.0[a]
	85	71.4[a]	0.902[a]	28.6[a]
	SEM	6.8	0.004	1.0
CG + PGA+ 40% PG	75	5.3[a]	0.899[a]	28.4[a]
	85	2.4[b]	0.899[a]	29.0[a]
	SEM	0.4	0.006	0.8
XG + 40% PG	75	4.7[a]	0.906[a]	26.1[b]
	85	2.0[b]	0.908[a]	31.4[a]
	SEM	0.4	0.004	0.8

a-b: means with the same letter by column are not different (P>0.05) due to relative humidity's within each coating control, coating with carrageenan, and coating with xanthan gum.
SEM: standard error of the mean

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Table 4.3 Mean values for mite infestation, water activity, and moisture content of cave aged Cheddar cheese cubes (2.54 cm x 2.54 cm x 2.54 cm) for untreated cubes, carrageenan, propylene glycol alginate, and propylene glycol coated cubes, and xanthan gum and propylene glycol coated cubes at different relative humidity and temperature combinations, after 14 d of incubation.

Treatment	Temperature (°C)	Relative humidity (%)	Mite count On cheese cubes (No.)	Water activity	Moisture Content (%)
Untreated cubes	10	75	21.8^{c}	0.881^{bc}	25.4^{c}
	10	85	35.5^{bc}	0.897^{abc}	26.1^{bc}
	15	75	64.5^{ab}	0.895^{abc}	20.2^{d}
	15	85	90.0^{a}	0.911^{a}	31.0^{ab}
	20	75	80.1^{a}	0.879^{c}	32.1^{a}
	20	85	88.7^{a}	0.899^{ab}	29.0^{abc}
	SEM		4.8	0.003	0.7
CG + PGA+ 40% PG	10	75	7.5^{a}	0.882^{b}	27.4^{bc}
	10	85	2.6^{cd}	0.905^{ab}	26.0^{c}
	15	75	4.5^{b}	0.901^{ab}	25.0^{c}
	15	85	1.7^{d}	0.893^{ab}	31.0^{ab}
	20	75	4.0^{bc}	0.914^{a}	33.3^{a}
	20	85	3.0^{bcd}	0.900^{ab}	30.0^{ab}
	SEM		0.3	0.004	0.5
XG + 40% PG	10	75	7.8^{a}	0.910^{a}	26.4^{c}
	10	85	3.2^{bc}	0.904^{a}	29.0^{bc}
	15	75	3.0^{bcd}	0.909^{a}	21.0^{d}
	15	85	1.4^{d}	0.917^{a}	32.0^{ab}
	20	75	3.5^{b}	0.899^{a}	31.0^{ab}
	20	85	1.5^{cd}	0.902^{a}	33.5^{a}
	SEM		0.3	0.003	0.6

a-d: means with the same letter by column are not different (P>0.05) due to relative humidity and temperature combination within each coating control, coating with carrageenan, and coating with xanthan gum.
SEM: standard error of the mean

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Table 4.4 Mean values for mite infestation, water activity, and moisture content of cave aged Cheddar cheese cubes (2.54 cm x 2.54 cm x 2.54 cm) for untreated nets, carrageenan, propylene glycol alginate, and propylene glycol netted cubes, and xanthan gum and propylene glycol netted cubes at different temperatures, when averaged over relative humidity after 14 d of incubation

Treatment	Temperature (°C)	Mite count On cheese cubes (No.)	Water activity	Moisture Content (%)
Untreated nets	10	16.0[b]	0.889[a]	25.7[b]
	15	63.0[a]	0.895[a]	25.7[b]
	20	69.0[a]	0.894[a]	31.5[a]
	SEM	4.13	0.005	1.23
CG + PGA+ 40% PG	10	7.0[a]	0.904[a]	26.6[b]
	15	5.1[ab]	0.902[a]	25.0[b]
	20	4.2[b]	0.903[a]	31.4[a]
	SEM	0.6	0.003	0.9
XG + 40% PG	10	6.4[a]	0.901[a]	28.1[ab]
	15	4.0[b]	0.906[a]	27.0[b]
	20	4.2[b]	0.902[a]	31.0[a]
	SEM	0.58	0.003	1.1

a-b: means with the same letter by column are not different (P>0.05) due to temperature within each net control, net with carrageenan, and net with xanthan gum.
SEM: standard error of the mean

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Table 4.5 Mean values for mite infestation, water activity, and moisture content of cave aged Cheddar cheese cubes (2.54 cm x 2.54 cm x 2.54 cm) for untreated nets, carrageenan, propylene glycol alginate, and propylene glycol netted cubes, and xanthan gum and propylene glycol netted cubes at different relative humidities, when averaged over temperature after 14 d of incubation

Treatment	Relative humidity (%)	Mite count On cheese cubes (No.)	Water activity	Moisture Content (%)
Net Control	75	52.7[a]	0.887[b]	26.0[b]
	85	45.8[a]	0.899[a]	30.0[a]
	SEM	3.34	0.004	1.0
Net with Carrageenan	75	7.1[a]	0.905[a]	26.2[b]
	85	3.7[b]	0.902[a]	29.1[a]
	SEM	0.47	0.003	0.7
Net with Xanthan Gum	75	6.4[a]	0.901[a]	27.3[b]
	85	3.3[b]	0.905[a]	30.0[a]
	SEM	0.47	0.003	0.9

a-b: means with the same letter by column are not different (P>0.05) due to relative humidity within each net control, net with carrageenan, and net with xanthan gum.
SEM: standard error of the mean

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Table 4.6 Mean values for mite infestation, water activity, and moisture content of cave aged Cheddar cheese cubes (2.54 cm x 2.54 cm x 2.54 cm) for untreated nets, carrageenan, propylene glycol alginate, and propylene glycol netted cubes, and xanthan gum and propylene glycol netted cubes at different relative humidity and temperature combinations after 14 d of incubation

Treatment	Temperature (°C)	Relative Humidity (%)	Mite count On cheese cubes (No.)	Water activity	Moisture Content (%)
Net Control	10	75	21.4^{b}	0.880^{cd}	23.5^{cd}
	10	85	10.4^{b}	0.898^{abc}	28.0^{bc}
	15	75	66.7^{a}	0.879^{d}	21.0^{d}
	15	85	59.1^{a}	0.911^{a}	30.5^{ab}
	20	75	70.0^{a}	0.901^{ab}	33.0^{a}
	20	85	68.0^{a}	0.888^{bcd}	30.0^{ab}
	SEM		2.34	0.002	0.7
Net with Carrageenan	10	75	8.7^{a}	0.913^{a}	25.2^{c}
	10	85	5.1^{bc}	0.894^{b}	28.0^{bc}
	15	75	7.2^{ab}	0.899^{b}	20.4^{d}
	15	85	3.0^{c}	0.906^{ab}	30.0^{ab}
	20	75	5.4^{b}	0.902^{ab}	30.0^{a}
	20	85	3.0^{c}	0.905^{ab}	29.7^{ab}
	SEM		0.33	0.002	0.5
Net with Xanthan Gum	10	75	8.1^{a}	0.904^{a}	27.0^{bc}
	10	85	4.6^{bc}	0.898^{a}	29.3^{ab}
	15	75	6.0^{ab}	0.902^{a}	24.0^{c}
	15	85	2.0^{d}	0.911^{a}	30.1^{ab}
	20	75	5.1^{bc}	0.897^{a}	31.2^{a}
	20	85	3.3^{cd}	0.906^{a}	30.2^{ab}
	SEM		0.33	0.002	0.6

a-d: means with the same letter by column are not different (P>0.05) due to relative humidity and temperature combination within each net control, net with carrageenan, and net with xanthan gum.
SEM: standard error of the mean

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Table 4.7 Difference-from-control sensory test results of cave aged cheddar cheese cubes (sliced into 1 cm thickness) treated by coatings with different food grade ingredients by experienced panelists (n = 6-8, 3 panels per trial) after 14 d of incubation

Condition	Treatment	Color	Aroma	Flavor	Texture	Moistness
	Blind Control	0.7^{a}	0.5^{a}	1.2^{a}	0.7^{a}	0.6^{a}
10°C 75% RH	**CG + PGA+ 40% PG**	0.9^{a}	0.6^{a}	1.3^{a}	1.0^{a}	0.8^{a}
	XG + 40% PG	0.7^{a}	0.4^{a}	1.0^{a}	0.7^{a}	0.5^{a}
	SEM	0.1	0.008	0.12	0.077	0.084
	Blind Control	0.5^{a}	0.5^{a}	0.8^{b}	0.4^{b}	0.4^{b}
15°C 75% RH	**CG + PGA+ 40% PG**	0.9^{a}	0.8^{a}	2.2^{a}	1.1^{a}	1.1^{a}
	XG + 40% PG	0.8^{a}	0.4^{a}	1.9^{a}	1.0^{a}	1.0^{a}
	SEM	0.08	0.07	0.13	0.08	0.051
	Blind Control	0.7^{a}	0.6^{a}	0.3^{b}	0.3^{b}	0.2^{c}
20°C 75% RH	**CG + PGA+ 40% PG**	0.9^{a}	0.9^{a}	1.5^{a}	0.8^{a}	1.1^{a}
	XG + 40% PG	0.7^{a}	0.6^{a}	1.0^{ab}	0.4^{b}	0.7^{b}
	SEM	0.1	0.08	0.14	0.07	0.07

PGA: Propylene glycol alginate, CG: Carrageenan, PG: Propylene glycol, XG: Xanthan gum

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

Scale for sensory evaluation against the labeled control: 0-no difference, 1-slight difference, 2-moderated difference, 3-large difference, 4-very large difference

SEM: standard error of the mean

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Table 4.8 Difference-from-control sensory test results of cave aged cheddar cheese cubes (sliced into 1 cm thickness) treated by coatings with different food grade ingredients by experienced panelists (n = 6-8, 3 panels per trial) after 14d of incubation

Condition	Treatment	Color	Aroma	Flavor	Texture	Moistness
10°C 85% RH	**Blind Control**	0.5[a]	0.4[a]	0.8[b]	0.6[a]	0.7[a]
	CG + PGA+ 40% PG	0.6[a]	0.4[a]	1.7[a]	0.9[a]	1.1[a]
	XG + 40% PG	0.9[a]	0.4[a]	2.0[a]	0.9[a]	1.2[a]
	SEM	0.083	0.066	0.112	0.1	0.1
15°C 85% RH	**Blind Control**	0.7[b]	0.6[b]	1.1[b]	1.0[a]	0.9[a]
	CG + PGA+ 40% PG	1.5[a]	1.3[a]	2.2[a]	1.3[a]	1.0[a]
	XG + 40% PG	1.0[ab]	1.2[a]	1.8[a]	1.1[a]	1.1[a]
	SEM	0.1	0.1	0.1	0.09	0.09
20°C 85% RH	**Blind Control**	0.8[a]	0.6[a]	0.8[b]	0.7[a]	0.7[a]
	CG + PGA+ 40% PG	1.2[a]	0.8[a]	2.1[a]	1.3[a]	1.2[a]
	XG + 40% PG	1.0[a]	0.7[a]	2.4[a]	1.2[a]	1.2[a]
	SEM	0.09	0.07	0.14	0.1	0.09

PGA: Propylene glycol alginate, CG: Carrageenan, PG: Propylene glycol, XG: Xanthan gum

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

Scale for sensory evaluation against the labeled control: 0-no difference, 1-slight difference, 2-moderated difference, 3-large difference, 4-very large difference

SEM: standard error of the mean

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Table 4.9 Difference-from-control sensory test results of cave aged cheddar cheese cubes (sliced into 1 cm thickness) treated by nettings with different food grade ingredients by experienced panelists (n = 6-8, 3 panels per trial) after 14 d of incubation

Condition	Treatment	Color	Aroma	Flavor	Texture	Moistness
10°C 75% RH	**Blind Control**	1.1[a]	0.5[a]	1.1[a]	0.7[a]	0.5[a]
	CG + PGA+ 40% PG	0.7[a]	0.3[a]	1.7[a]	0.8[a]	0.8[a]
	XG + 40% PG	1.0[a]	0.4[a]	1.3[a]	0.8[a]	0.7[a]
	SEM	0.1	0.06	0.12	0.08	0.08
15°C 75% RH	**Blind Control**	0.8[a]	0.4[a]	1.1[b]	0.7[a]	0.5[a]
	CG + PGA+ 40% PG	0.8[a]	0.7[a]	1.7[a]	0.8[a]	0.8[a]
	XG + 40% PG	0.8[a]	0.8[a]	1.3[ab]	0.8[a]	0.7[a]
	SEM	0.1	0.07	0.12	0.08	0.08
20°C 75% RH	**Blind Control**	0.4[a]	1.0[a]	*	*	*
	CG + PGA+ 40% PG	0.8[a]	1.0[a]	*	*	*
	XG + 40% PG	0.8[a]	1.1[a]	*	*	*
	SEM	0.08	0.1	*	*	*

PGA: Propylene glycol alginate, CG: Carrageenan, PG: Propylene glycol, XG: Xanthan gum

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

Scale for sensory evaluation against the labeled control: 0-no difference, 1-slight difference, 2-moderated difference, 3-large difference, 4-very large difference

*Note: Sensory analysis could not be conducted due to presence of excess mold

SEM: standard error of the mean

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Table 4.10 Difference-from-control sensory test results of cave aged cheddar cheese cubes (sliced into 1 cm thickness) treated by nettings with different food grade ingredients by experienced panelists (n = 6-8, 3 panels per trial) after 14 d of incubation

Condition	Treatment	Color	Aroma	Flavor	Texture	Moistness
	Blind Control	0.7[a]	0.5[a]	0.8[b]	0.5[a]	0.5[a]
10°C 85% RH	**CG + PGA+ 40% PG**	0.7[a]	0.6[a]	1.3[ab]	0.7[a]	0.8[a]
	XG + 40% PG	0.8[a]	0.5[a]	1.5[a]	0.9[a]	0.8[a]
	SEM	0.09	0.07	0.1	0.08	0.08
	Blind Control	1.1[a]	0.6[a]	0.7[a]	0.6[a]	0.5[a]
15°C 85% RH	**CG + PGA+ 40% PG**	0.4[a]	0.4[a]	0.8[a]	0.7[a]	0.4[a]
	XG + 40% PG	*	*	*	*	*
	SEM	0.1	0.1	0.2	0.2	0.1
	Blind Control	0.8[a]	0.9[a]	*	*	*
20°C 85% RH	**CG + PGA+ 40% PG**	1.0[a]	1.3[a]	*	*	*
	XG + 40% PG	1.0[a]	0.9[a]	*	*	*
	SEM	0.12	0.13	*	*	*

PGA: Propylene glycol alginate, CG: Carrageenan, PG: Propylene glycol, XG: Xanthan gum

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

Scale for sensory evaluation against the labeled control: 0-no difference, 1-slight difference, 2-moderated difference, 3-large difference, 4-very large difference

*Note: Sensory analysis could not be conducted due to presence of excess mold

SEM: standard error of the mean

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CHAPTER V

CONCLUSIONS

Mite reproduction of *T. putrescentiae* was not controlled on cave aged Cheddar cheese cubes (2.5 cm x 2.5 cm x 2.5 cm) at any temperature and relative humidity combinations with untreated nets and untreated cubes. However, storage of cubes at 10°C at both 75% RH and 85% RH slowed mite growth, indicating that aging cheese at 10ºC or less can help reduce growth. The CG + PGA+ 40% PG and XG and 40% PG for both coating and net treatments inhibited the growth of mites. Although all temperature and relative humidity combinations affected the overall sensory quality of the cheese, the 10°C treatment at 75% RH did not affect the sensory quality for both coatings and nettings, indicating that a lower temperature of aging cheese along with treatments did not impact the sensory quality. In addition, use of nets lessened the impact of the food grade coatings on the sensory properties of the Cheddar cheese. Further research is needed to evaluate ways to use these food grade coatings without impacting the sensory properties of Cheddar cheese during aging.

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