

Antimicrobial effects of multifunctional ingredients with potential application for ready to eat
meat and poultry products

by

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Abstract

Consumer demand for clean-label and 'all natural' food products has created the need to investigate antimicrobials derived from natural sources. Multifunctional ingredients are food additives that have multiple properties to reduce fat, limit salt, retard oxidation, increase water-holding capacity and inhibit bacterial growth in foods. Multifunctional ingredients that exhibit antimicrobial effects in meat and poultry products can facilitate consumers demand for clean and 'all natural' labels while reducing foodborne illness risk.

Previous scientific research has shown that plant essential oils are known to contain active components to prevent oxidation in meat products, but emerging data have shown that these plant-based ingredients also contain antimicrobial properties. Plant essential oils such as basil oil has shown limited *Salmonella* Enteritidis inhibition in meat model systems and thyme oil has shown *Listeria monocytogenes* inhibition in low fat beef hotdogs. Intrinsic and extrinsic parameters of meat systems can alter the antimicrobial efficacy of plant essential oils. Although antimicrobial effects were observed with plant essential oils, effective usage levels may be limited to sensory characteristics in certain meat and poultry products.

Natural extracts have shown potential antimicrobial properties in meat and poultry applications. Rosemary extract has been shown to suppress the growth of Enterobacteriaceae, *Pseudomonas*, and yeast and molds in fresh sausage. Grapefruit seed extract has shown inhibition against *Campylobacter jejuni* in poultry skin and meat models and *E. coli* O157:H7 in moisture enhanced beef homogenate models. The addition green tea extract in ground beef has been shown to reduce *D*-values while cooking and inhibit outgrowth of *C. perfringens* spores during extended chilling of cooked ground beef. Grape seed extract has been shown to

reduce *Listeria monocytogenes*, *E. coli* O157:H7 and *Salmonella* Typhimurium populations in cooked lean ground beef stored for 9 days at 4°C.

Scientific research findings for plant essential oils and extracts confirm that multifunctional ingredients are relevant to meat and poultry products as potential food additives to control undesirable pathogen and spoilage bacteria while meeting consumer demand for natural, clean-label ingredients.

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Chapter 1 - Definitions

The following terms used in the context of this report are defined below.

‘Clean label’:

Foods that have simple ingredients that are recognized by consumers and perceived as being derived from a nonchemical source (McDonnell 2013).

Minimally processed:

As defined by the USDA FSIS (2005), minimally processed is “a traditional processes used to make food edible or to preserve it or make it safe for human consumption, (e.g., smoking, roasting, freezing, drying, and fermenting) or a physical processes that does not fundamentally alter the raw product and/or that only separate a whole, intact food into component parts, (e.g., grinding meat, separating eggs into albumen and yolk, and pressing fruits to produce juices)”.

Multifunctional Ingredients:

Direct food additives that have more than one purpose in a food product.

‘Natural’:

As defined by the USDA FSIS Food Standards and Labeling Policy Book (USDA FSIS 2005)

- 1) The (food) product does not contain any artificial flavor or flavoring, coloring ingredient, or chemical preservative (as defined in 21 C.F.R. §101.22), or any other artificial or synthetic ingredient; and
- 2) The (food) product and its ingredients are not more than minimally processed.

Chapter 2 - Introduction

Food safety is an important concern to consumers. In a food and health survey conducted by the International Food Information Council Foundation (2015), consumer confidence in the United States food supply decreased from 78% in 2012 to 61% in 2015. Ready-to-eat (RTE) meat products are a popular meat item in the United States that can be a source of foodborne illness (USDA FSIS 2013). Ready-to-eat meat products potentially can be contaminated post-lethality prior to packaging by foodborne pathogens such as *Listeria monocytogenes* (Beresford and others 2001). *Listeria monocytogenes* is a foodborne pathogen concern for RTE meat products because it causes listeriosis, a severe foodborne disease that is particularly harmful to pregnant women, newborns and adults with weakened immune systems (Ahmed 2015). Currently, many United States meat and poultry processors utilize antimicrobial treatments such as sodium lactate or potassium lactate-sodium diacetate to suppress the outgrowth of *L. monocytogenes* should post lethality contamination occur (McDonnell and others 2013). Nitrite is a common ingredient that is responsible for the distinctive color and flavor of cured meats and is also utilized to control the outgrowth of harmful pathogenic bacteria such as *L. monocytogenes*, *Clostridium perfringens*, and *C. botulinum* (Golden and others 2014).

In conjunction to food safety, consumers demand that RTE foods have extended shelf life and maintain fresh quality (Marth 1998). Spoilage microorganisms can cause quality defects in meat such as off-odors, off-flavors, discoloration and gas production. These defects degrade the shelf life of meat products and inherently lower the product's market value. Ready-to-eat processed meats typically have an extended shelf life, which require additional

processes or ingredients to be incorporated to inhibit the growth of spoilage bacteria. The theory of hurdle technology states that a multi-targeted approach of “intelligently applied gentle (food preservation) hurdles” will have a synergistic effect on food preservation (Leistner 2000). According to the United States Department of Agriculture Food Safety Inspection Service (USDA FSIS) (2011), there are two types of parameters affecting the growth of microorganisms in food products: extrinsic and intrinsic. Extrinsic parameters are properties of the environment (processing and storage) that exist outside the food product that affect both the food and associated microorganisms. Examples of extrinsic parameters for RTE meat and poultry products include storage temperature, relative humidity, presence/concentration of gases, and presence/activities of other microorganisms (USDA FSIS 2011). Spoilage microorganisms associated with refrigerated RTE meat products are predominantly lactic acid bacteria consisting of *Lactobacillus* spp. and *Leuconostoc* spp. in anaerobic storage conditions whereas *Pseudomonas* spp., *Bacillus* spp., *Micrococcus* spp. and *Lactobacillus* spp. dominate in aerobic conditions (Borch 1996). Intrinsic parameters are properties that exist within a food product, which can be altered with the addition of ingredients. Examples of intrinsic parameters for RTE meat and poultry products include pH, moisture content, water activity, oxidation-reduction potential, nutrient content and antimicrobial constituents (USDA FSIS 2011). By using hurdle technology, the use of multiple extrinsic and intrinsic parameters can enhance the antimicrobial effect against bacteria.

Consumers view ‘natural’ food products as foods that are fresh, have only a few, simple ingredients and have been subjected to minimal processing (Becker 2012). Lu Ann Williams, Director of Innovation at Innova Market Insights, prefers the term ‘clear-label’ to ‘clean-label’

because it relates the concept of transparency (Kuhn 2015). The definition of clean-label continues to broaden every year as consumers lump many characteristics together such as no artificial colors, no additives, organic, or Fair Trade. “Clean—or clear label as we like to call it—has moved past being a trend. It is a new rule. Companies will have to do what they can to clean up labels or be as transparent as they can moving forward” (Kuhn 2015).

Traditional deli meat items often contain synthetic antimicrobials that inhibit growth of pathogens and spoilage bacteria, but these additives are not permitted in meat and poultry products bearing natural claims. The federal governing body for meat products, USDA FSIS, has guidelines for food additives that can be used in natural meat and poultry products (USDA FSIS 2015). According to McDonnell and others (2013), “Nitrate and nitrite are considered chemical preservatives under the definition of ‘natural’ and are specifically listed as prohibited ingredients for products following organic labeling criteria”. Ready-to-eat meat and poultry items with natural claims are steadily increasing in popularity on grocery store shelves as alternatives to traditional deli products that contain synthetic preservatives. Consumer interest and market growth for natural, organic and clean label foods have increased by 20 to 22% of the annual market share from 1997 to 2007 (McDonnell and others 2013). Furthermore, a recent survey conducted by Food Marketing Institute & American Meat Institute (2014) revealed that 34% of shoppers purchased natural or organic meats in the past three months leading the surveyors to conclude that this trend is increasing.

Controlling extrinsic factors of a meat and poultry product may not be sufficient to limit pathogens and unwanted spoilage organisms. Uncured or traditionally cured RTE meat

and poultry products without additional antimicrobial ingredients can support growth of *L. monocytogenes* during cold storage at 4°C, should post-lethality contamination occur (McDonnell and others 2013). Jeff Sindelar, Associate Professor and Extension Meat Specialist at the University of Wisconsin-Madison stated, "Most ingredients and technologies that serve as antimicrobials—ingredients that can improve the safety by either suppressing, inhibiting, or destroying any pathogenic bacteria—are not able to be used in products labeled 'natural' and 'organic' "(Day 2013). The natural ingredient limitations designated by the USDA FSIS makes it challenging for processors to meet consumer demand for safe, high quality, natural RTE meat products. It is apparent that manipulating intrinsic parameters must be considered when developing RTE meat products to reduce the likelihood of pathogen outgrowth.

The objective of this report is to review the regulatory limitations of antimicrobial ingredients in natural meat and poultry products, evaluate why certain ingredients are approved to control the outgrowth of pathogens in natural meat and poultry products as an incidental effect, and to review scientific literature on emerging natural ingredients that have antimicrobial properties which are potentially relevant to natural RTE meat applications.

Chapter 3 - Regulatory Considerations for the Term Natural

It is important to understand federal labeling regulations to determine why certain food ingredients are allowed in natural meat products. In a 1991 Federal Register notice (U.S Food and Drug Administration 1991), the U.S. Food and Drug Administration (FDA) requested comments from the public to determine whether it was appropriate to define the term 'natural' or ban such claims entirely on the ground that they are false or misleading. After reviewing the comments, FDA decided not to further define the term natural and would "maintain its current policy (FDA 1991) to not restrict the use of the term natural except for added color, synthetic substances, and flavors as provided in 21 C.F.R. §101.22" (FDA 1993). "Additionally, the agency will maintain its policy regarding the use of natural as meaning that nothing artificial or synthetic (including all color additives regardless of source) has been included in, or has been added to, a food that would not normally be expected in the food" (FDA 1993). More recently in a 2015 Federal Register notification (FDA 2015b), FDA has once again asked the public if 'natural' should be defined in the context of food labeling due to three citizen petitions asking the FDA to define 'natural' and one citizen petition asking the FDA to prohibit the term 'natural'. The FDA has not published a formal definition for the term 'natural' and the only codified reference for 'natural' is in the FDA natural flavor regulation (FDA 2016; Hibbert 2007).

The USDA FSIS collaborates with the FDA on regulatory topics, but has been inconsistent with respect to its view of the term natural for meat and poultry products (Hibbert 2007). For example, some natural ingredients, such as salt, have dual functions as flavorings and natural preservatives (Sebranek and Bacus 2007). Furthermore, beets, which are a natural source of

pigment, are disallowed as coloring agents in natural products, while paprika, also a natural source of pigment, is considered acceptable by USDA FSIS as a seasoning ingredient in certain meat products because it has a primary function for flavor although it may impart color in a finished meat product (McKeith 2014).

The USDA FSIS released the first policy guidance regarding the term ‘natural’ in the form of Standards and Labeling Policy Memorandum (Memo) 055 dated November 22, 1982 (USDA FSIS 2006). In 2003, USDA FSIS rescinded the Standards and Labeling Policy Memo 055 and included the definition of ‘natural’ in the Food Standards and Labeling Policy Book (USDA FSIS 2005). The USDA FSIS Food Standards and Labeling Policy Book was updated in 2005 and remains the most recently written collection of records on the term ‘natural’ for meat and poultry. In this book (USDA FSIS 2005), ‘natural’ is defined as: 1) the product does not contain any artificial flavor or flavoring, coloring ingredient, or chemical preservative (as defined in 21 C.F.R. §101.22), or any other artificial or synthetic ingredient; and 2) the product and its ingredients are not more than minimally processed.” In the USDA FSIS Food Standards and Labeling Policy Book, the USDA FSIS (2005) defined minimal processing as “a) traditional processes used to make food edible or to preserve it or make it safe for human consumption, (“e.g., smoking, roasting, freezing, drying, and fermenting”); or b) physical processes that do not fundamentally alter the raw product and/or that only separate a whole, intact food into component parts, (“e.g., grinding meat, separating eggs into albumen and yolk, and pressing fruits to produce juices”)”. Relatively severe processes, such as solvent extraction, acid hydrolysis, and chemical bleaching, would be considered more than minimal processing (USDA FSIS 2005).

Also outlined in the USDA FSIS Food Standards and Labeling Policy Book (USDA FSIS 2005), the USDA FSIS states, “All products claiming to be natural or a natural food should be accompanied by a brief statement which explains what is meant by the term natural, i.e., that the product is a natural food because it contains no artificial ingredients and is only minimally processed. This statement should appear directly beneath or beside all natural claims or, if elsewhere on the principal display panel; an asterisk should be used to tie the explanation to the claim” (USDA FSIS 2005).

To explain the complexity of natural claims within the USDA FSIS, a regulatory approval that was retracted from the Food Standards and Labeling Policy Book is discussed. According to USDA FSIS in the Federal Register (USDA FSIS 2006), the USDA FSIS modified its longstanding 1982 Policy Memo 055 in 2005 to acknowledge, “sugar, sodium lactate (from a corn source) [at certain levels] and natural flavorings from oleoresins or extractives are acceptable for ‘all natural’ claims.” However, on October 9, 2006, Hormel Foods submitted a petition to USDA FSIS for rulemaking to codify a definition of natural for meat and poultry inspection regulations (USDA FSIS 2006). Public comments expressed concern about whether the 2005 USDA FSIS judgment that sodium lactate, at levels approved for flavoring, was consistent with the meaning of natural (USDA FSIS 2006). In response, the USDA FSIS (2009) stated that the information indicated that sodium lactate (as well as potassium lactate and calcium lactate) may provide an antimicrobial effect at levels approved for flavoring. The USDA FSIS concluded in 2006 that listing sodium lactate (from a corn source) for natural meat and poultry products “may have been in error” (USDA FSIS 2006). In the same publication (USDA FSIS 2006), USDA FSIS modified the natural claim reference to omit sodium lactate in the 2005 Policy Book. According to USDA

FSIS (2005), “the current entry in the Policy Book provides that use of sodium lactate (or any ingredient known to have multiple technical effects in products labeled as natural) will be evaluated on a case-by-case basis to determine whether the intended use, level of use, and technical function are consistent with the original 1982 policy.”

Due to the complex regulatory hurdles for natural claims, the category of clean-label has emerged as one solution to meet consumers labeling expectations. ‘Clean-label’ foods are not restricted to USDA FSIS definitions of natural or organic but have simple ingredients that are recognized by consumers and are perceived as being derived from a nonchemical, non-synthetic source (McDonnell 2013). These ingredients include vinegar, flavorings, cultured sugars or dairy ingredients, or ingredients derived from plant material (McDonnell 2013). There is no legal or regulatory definition for ‘clean-label’ as this term is defined by consumers and stakeholders such as retailers (Hutt and Sloan 2015). Hutt and Sloan (2015) also stated that, “It is generally agreed that ‘clean-label’ ingredients are minimally processed, devoid of artificial flavors, artificial colors, synthetic additives and absent of any unexpected allergens.” Clean-label ingredients intended for use in meat and poultry products must still be approved case-by-case by the USDA FSIS pre-market approval process (USDA FSIS 2015).

The USDA FSIS categorizes direct food additives as antimicrobials, antioxidants, binders and flavoring agents (USDA FSIS 2016). Multifunctional ingredients can be utilized in foods and in particular, RTE meat and poultry products, to reduce and simplify a label. One of the most widely used multifunctional ingredients in fresh and RTE meat and poultry products is rosemary extract (Madsen and Bertelsen 1995). Rosemary contains phenolic di-terpenoid compounds,

which serve as strong antioxidants in meat and poultry products (Klancnik and others 2009). The USDA FSIS generally views spice extracts and flavoring constituents as natural ingredients used for flavoring purposes (Sebranek and others 2012). This allows a processor to claim natural flavors rather than the common name. The FDA defined natural flavor or natural flavoring as “...the essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any product of roasting, heating or enzymolysis, which contains the flavoring constituents derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or similar plant material, meat, seafood, poultry, eggs, dairy products, or fermentation products thereof, whose significant function in food is flavoring rather than nutritional. Natural flavors include the natural essence or extractives obtained from plants listed in §§182.10, 182.20, 182.40, and 182.50 and part 184 of this chapter, and the substances listed in §172.510 of this chapter” (FDA 2015a). The FDA definition of ‘natural flavor’ supports the use of multifunctional ingredients in foods as long as the significant function of the ingredient is flavoring.

As previously mentioned, in 21 CFR part 101.22, natural extracts and plant essential oils derived from natural sources meet the FDA labeling guidelines for natural flavoring and previous research has indicated that these food additives have significant bacterial inhibition (Fernandez-Lopez 2005; Hegggers and others 2002; Kim and others 2004; Pandit and Shelef 1994). In response to an advanced notice of proposed rulemaking by USDA FSIS (USDA FSIS 2009), public comments suggested USDA FSIS distinguish ingredients used for their antimicrobial effects to inhibit growth of pathogens such as *L. monocytogenes*, from those used for preservative effects. Chemical preservatives are defined in 9 CFR 301.2 as “any chemical

that, when added to a food, tends to prevent or retard deterioration of food, and this effect is achieved by preventing the outgrowth of microorganisms that produce off-flavors and discolor food as the food ages” (USDA FSIS 2009). The USDA FSIS has stated that firms seeking USDA FSIS approval for natural claims in the labeling of products that include a multifunctional ingredient would be subject to a case-by-case approval and would need to substantiate the claim with, among other evidence, a showing that the ingredient is not being used to extend the shelf life of the product (USDA FSIS 2009).

Based on a literature review of regulatory limitations for natural food additives that have antimicrobial properties, there is no codified policy for these ingredients in natural meat and poultry products. Ready-to-eat meat and poultry product processors have options depending on their labeling needs. Adhering to a clean-label position, such as a ‘no artificial ingredients’ claim, may be easier than obtaining a ‘natural’ claim but the definition of ‘clean-label’ is open to the ever-changing scrutiny of consumer opinion. Multifunctional ingredients allow processors to meet regulatory labeling guidelines for ‘natural’ foods while providing meat and poultry products with reduced risk of foodborne illness.

There have been numerous scientific studies conducted to evaluate potential antimicrobial ingredients from natural sources for food applications. In general, natural antimicrobials are derived from animal, plant, and microbial sources (Davidson and others 2013). As a general rule, an antibacterial agent should be bactericidal and non-toxic (Hegggers and others 2002). Antimicrobial agents that kill bacteria are referred to as bactericidal, while those that prevent bacteria from multiplying are bacteriostatic (Anderson and others 2012).

Bactericidal agents must kill >99.9% of the bacteria to be considered bactericidal (Pankey and Sabath 2004). Natural sources of antibacterial agents have been researched to understand their impact using in-vitro studies but little published research is available for *in-situ* applications using RTE meat. This report will review six potential multifunctional ingredients; four extracts and three plant essential oils.

Chapter 4 - Antimicrobial Properties of Multifunctional Ingredients

As mentioned previously, natural flavors are the most commonly used multifunctional food additive due to the label-friendly perception by consumers. Within the category of natural flavors, natural extracts are of particular interest as these food additives have shown microbial inhibition in several studies using *in vitro* experiments (Reagor and others 2002; Cvetnić and Vladimir-Knežević 2004; Ahn and others 2003). Many natural extracts exhibit antimicrobial properties, including rosemary extract (Del Campo and others 2000; Karamanoli and others 2000), grapefruit seed extract (Reagor and others 2002; Xu and others 2007), green tea extract (Juneja and others 2007; Kim and others 2004) and grape seed extract (Ahn and others 2003). The bioactive compounds responsible for the antimicrobial activities of natural extracts are polyphenolic components, bound to plant sugars as glycosides, and include flavonoids, terpenoids, vitamins, minerals, carotenoids, and phytoestrogens (Merken 2001; Rodriguez Vaquero and others 2010). Plant essential oils, such as basil oil, rosemary oil, and thyme oil, have also shown to inhibit bacteria growth (Gill and others 2002; Rattanachaiakunsopon and Phumkhachorn 2010; Singh and others 2003; Pandit and Shelef 1994). Each of these multifunctional antimicrobials from natural sources will be addressed in detail.

Rosemary Extract

Rosemary extract has been shown to retard lipid oxidation and inhibit microbial growth in meat applications (Nissen and others 2004; Piskernik and others 2011; Georgantelis and others 2007). Water-miscible, oil-miscible, and oil-and-water miscible rosemary were screened for antimicrobial properties using an agar diffusion method (Fernandez-Lopez 2005). Fernandez-Lopez (2005) reported oil-miscible rosemary exhibited inhibitory effects against 11

foodborne spoilage organisms sourced from vacuum-packaged meat. Furthermore, oil miscible rosemary expressed an inhibition zone of 25 mm in the agar diffusion assay for *L. monocytogenes* whereas water miscible rosemary and oil and water miscible rosemary had inhibition zones of 19.5 mm and 15.4 mm, respectively (Fernandez-Lopez 2005). Previous research concluded that the phenolic di-terpenoids are the main antimicrobial compounds, which are non-polar fractions of rosemary extract (Del Campo and others 2000; Karamanoli and others 2000). Furthermore, Davidson and others (2013) reported that gram-positive microorganisms are generally more susceptible to nonpolar phenolic compounds than gram-negative microorganisms. *Listeria monocytogenes* is a gram-positive pathogen commonly linked as the causative organism involved in foodborne illness in RTE meats (Farber and Peterkin 1991). Gram-positive bacteria do not contain an outer membrane within their cell membrane, which might allow the cell to be more sensitive to external environmental changes such as temperature, pH, natural extracts and composition of meat as a substrate (Shelef and others 1980; Chung and others 1989). Unlike gram-positive bacteria, lipopolysaccharide proteins in the cell membrane of gram-negative bacteria may provide a barrier that does not allow antimicrobials to pass through the cell wall (Juven and others 1994). Certain natural compounds might change the functions of the bacterial cell membrane that restrict certain antimicrobials (Ahn 2003).

In a study performed by Piskernik and others (2011), rosemary extract was evaluated to determine the antimicrobial effect against *Campylobacter jejuni* in a chicken meat juice model. Rosemary extract tested at the highest concentration level of 0.31 mg/ml efficiently reduced *C. jejuni* at an initial inoculation level of approximately 10^3 CFU/ml to a non-detectable level after

24 hours at 42°C. Additionally, an initial inoculum level of approximately 3.0 log CFU/ml *C. jejuni* was reduced in the sample of chicken meat juice that contained 0.31 mg/ml rosemary extract to non-detectable levels when stored at chilling temperatures of 8°C for 120 hours. Piskernik and others (2011) stated that rosemary extract at 0.08 mg/ml and 0.16 mg/ml did not have any effect on *C. jejuni* at medium and high inoculum levels of approximately 5 log CFU/ml and 7 log CFU/ml, respectfully.

Georgantelis and others (2007) conducted a study to evaluate rosemary extract individually and in combination with chitosan as a natural antimicrobial in 25% fat fresh pork sausage stored in aerobic packaging at 4°C for 20 days. The authors found that sausage containing 260 ppm rosemary extract had a lactic acid bacteria population of 6.52 log CFU/g and this was lower ($P \leq 0.05$) than the control sausage which did not contain treatment rosemary extract and had a population of 7.01 log CFU/g after 5 days at 4°C (Georgantelis and others 2007). Georgantelis and others (2007) reported that after the 20 days of storage at 4°C, sausage containing 260 ppm rosemary extract had a *Enterobacteriaceae* population of 4.08 log CFU/g compared to a control sausages that had a *Enterobacteriaceae* population of 5.38 log CFU/g. Additionally, the sausage containing 260 ppm rosemary extract had a pseudomonad population of 7.06 log CFU/g, which was lower ($P \leq 0.05$) than control sausage with 7.54 log CFU/g after the 20 day storage period (Georgantelis and others 2007). Regardless of the presence of absence of rosemary extract, the sausages had reached an index of spoilage at 20 days of refrigerated storage. Georgantelis and others (2007) also evaluated incorporation of 1% chitosan in pork sausage. They found that inclusion of 1% chitosan in fresh pork sausage led to total viable counts of 6.01 log CFU/g after 15 days at 4°C compared to pork sausage

containing rosemary extract treatment and the control, which had total viable counts of 7.78 CFU/g and 7.89 CFU/g, respectfully. Chitosan is a modified, natural biopolymer derived by deacetylation of chitin, a major component of the shells of crustacean (No and others 2007). Although the antimicrobial effect of rosemary extract alone was less than that of chitosan, a combination of rosemary and chitosan resulted in a total viable count of 5.51 CFU/g. Georgantelis and others (2007) suggested that there may be a synergistic effect between rosemary extract and chitosan as this combination had the greatest bacteriostatic effect for *Enterobacteriaceae*, *pseudomonas*, lactic acid bacteria, and yeast and molds at day 15.

Previous research has indicated that rosemary extract is an efficient natural antioxidant and antimicrobial for use in meat applications (Madsen and Bertelsen 1995; Piskernik and others 2011). According to Nychas and others (2008), off odors in aerobic fresh meat are perceivable to consumers when the total bacteria count around 10^7 CFU/g. The study conducted by Georgantelis and others (2007) is not practical for meat and poultry products, as rosemary extract may not have shown a shelf life extension large enough to justify the addition of the natural extract. Rosemary extract can be considered a multifunctional ingredient due to the flavor, antioxidant and antimicrobial properties that it contributes to food. Although antimicrobial research has been conducted in various meat application models, further research is needed to evaluate its efficacy in RTE deli meat and poultry applications.

Grapefruit Seed Extract

An emerging natural extract for microbial inhibition is grapefruit seed extract (GSE). Although GSE is not specifically listed in 21 CFR part 182, grapefruit extract, which is listed in 21 CFR part 182.20, is the extract of the seeds of the grapefruit, *Citrus paradisi Macf.* (Winter

2009). Grapefruit seed extract has been shown to possess antibacterial, antifungal, antiviral and anti-parasite properties (Hegggers and others 2002, Reagor and others 2002, Ionescu and others 1990; Trillini 2000).

In a study conducted by Xu and others (2007), GSE provided an antimicrobial effect in minimally processed vegetables. Grapefruit seed extract at 0.1% in a water wash treatment reduced populations ($P \leq 0.05$) of *Salmonella* spp. over an 8 day storage period by 3.45 log CFU/g on whole cucumber and 1.20 log CFU/g on lettuce compared to a control wash treatment of water only in an inoculation (Xu and others 2007). In the same study, *L. monocytogenes* was reduced by 2.16 log CFU/g on whole cucumber and 1.00 log CFU/g on lettuce over the 8 day storage period. Xu and others (2007) also reported a 0.1% GSE wash treatment could delay spoilage from aerobic psychrotrophic bacteria for 2 days compared to the water only wash treatment. These results are aligned with previous studies that found GSE is an effective broad-spectrum bactericide (Reagor and others 2002), fungicide (Ionescu and others 1990) and antiviral and antiparasitic (Trillini 2000) natural extract.

The natural antimicrobial effect of GSE has been debated recently due to several reports (Sakamoto and others 1996; von Woedtke 1999) that claimed GSE contained synthetic compounds that inhibit bacteria. Commercial GSE that contains synthetic compounds would not be considered 'natural' if chemical preservatives (as defined in 21 CFR 101.22) are found in a food (USDA FSIS 2005). Sakamoto and others (1996) reported two chemicals, Methyl-p-hydroxybenzoate and 2,4,4'-trichloro-2'-hydroxydiphenylether (triclosan), were present in commercially available GSE compared to an ethanol extract of grapefruit seed. In a separate

study (von Woedtke 1999), six GSE from commercial sources were examined for antimicrobial properties. Using thin layer chromatography, the preservative benzethonium chloride was present in five extracts that showed inhibitory effects against spoilage bacteria (von Woedtke 1999). According to the U.S. National Library of Medicine (2016), benzethonium chloride is a quaternary ammonium surfactant that is used as an antiseptic and disinfectant against a broad spectrum of bacteria, fungi, molds and viruses. However, von Woedtke (1999) reported that lab-made extracts from seeds and pulp of grapefruit contained no antimicrobial activity. Cvetnić and Vladimir-Knežević (2004) challenged the studies (Sakamoto and others 1996; von Woedtke 1999) that stated that ethanoic extracts of grapefruit seeds did not exhibit antimicrobial effects. Self-made 33% (*m/V*) ethanoic extract of grapefruit seeds was screened against 20 bacterial strains and 10 yeast strains by the agar diffusion method (Cvetnić and Vladimir-Knežević 2004). To verify that the antimicrobial effect was not due to the ethanol used to prepare the self-made 33% (*m/V*) ethanoic extract of grapefruit seeds, Cvetnić and Vladimir-Knežević (2004) tested a 70% ethanol treatment, which did not show any zones of inhibition. In the agar diffusion assay, the self-made GSE extract expressed inhibition against all tested gram positive bacteria, but exerted no inhibition on the growth of the tested gram negative bacteria. Grapefruit seed extract exhibited the largest zones of inhibition for *L. monocytogenes* (16 mm), *Streptococcus faecalis* (15 mm) and *Bacillus subtilis* (14 mm). In a broth dilution susceptibility test, self-made GSE at a concentration of 16.5% (*m/V*) showed bactericidal/fungicidal effects against all microbial strains evaluated. The self-made GSE expressed bactericidal effects against most tested microorganisms, including gram positive and gram negative bacteria, at 8.25% (*m/V*) with the exception of *B. cereus*, *B. subtilis*, *Sarcina flava*

and *E. coli*. The self-made GSE at 8.25% (m/V) expressed bacteriostatic effects against *B. cereus*, *B. subtilis*, *Sarcina flava* and *E. coli* (Cvetnić and Vladimir-Knežević 2004). Although the results from the study conducted by Cvetnić and Vladimir-Knežević (2004) showed that self-made ethanoic GSE was less efficacious than commercial GSE preparations studied by Sakamoto and others (1996) and von Woedtke (1999), the self-made ethanoic GSE presented consistent antimicrobial effects. The results by Cvetnić and Vladimir-Knežević (2004) illustrate that GSE from a pure ethanoic extraction process inhibits gram-positive and gram-negative bacteria.

In a study conducted by Riedel and other (2009), GSE was evaluated against *C. jejuni* in a chicken skin and meat model. Thirty-five ml core samples were aseptically cut from *Campylobacter*-negative chickens. The skin samples were inoculated with a 50 µl sample of *C. jejuni* and air dried for 20 minutes to allow bacteria to adhere to the skin surface. Initial population count of *C. jejuni* on in the skin before treatment with GSE was 5.37 log CFU/ml. The inoculated skin samples were dipped for 1 minute in an antimicrobial treatment of 1.6% (wt/vol) GSE. The skin samples treated with GSE for 1 minute were homogenized by stomaching for 2 minutes with 100 ml of buffered peptone. The aliquot was serially diluted and plated on dried modified Abeyta-Hunt-Bark agar supplemented with triphenyl-terazolium chloride. The plates were incubated in aerobic conditions at 42°C for 48 hours. The skin sample treated with 1.6% (wt/vol) GSE for 1 minute resulted in a 3.05 log CFU/ml reduction of *C. jejuni*. Samples stored at 5°C for 24 hours had *C. jejuni* population reductions of 2.15 log CFU/ml compared with the reduction determined immediately after the 1 minute dip of GSE (3.05 log CFU/ml). The lower reduction after 24 hours indicates that GSE did not have bacteriostatic effects against *C. jejuni* in a chicken skin model.

In a moisture-enhanced beef model system, GSE was evaluated to reduce surface contamination of *E. coli* O157:H7 (Ko and others 2015). In an inoculated pathogen study, 0.5% GSE was added to fresh beef knuckles that were enhanced with water, salt and sodium tripolyphosphate. The beef knuckles were homogenized and homogenate was filtered through cheesecloth. The filtered homogenate was inoculated with approximately 3 log CFU/g of *E. coli* O157:H7. The sample containing 0.5% GSE immediately had a reduction in the initial population of *E. coli* O157:H7 from approximately 3 log CFU/g to non-detectable levels and after 24 hours stored at 15°C the *E. coli* O157:H7 population remained non-detectable (Ko and others 2015). Furthermore, Ko and others (2015) reported 0.5% GSE in the beef homogenate model had immediate bactericidal effects on aerobic bacterial populations and bactericidal effects over a 48 hour storage period at 15°C.

Shin and others (2012) reported that initial inoculation populations for *L. monocytogenes* of 7.0 log CFU/g and *E. coli* of 6.4 log CFU/g on bacon wrapped with red algae film containing 1% GSE were reduced to 6.8 log CFU/g and 5.8 log CFU/g, respectively, after 3 days at 4°C. *Listeria monocytogenes* and *E. coli* populations eventually increased to 7.6 log CFU/g and 6.6 log CFU/g, respectively, during the 15 day storage at 4°C indicating that GSE was not bacteriostatic after the population reduction on day 3. Shin and others (2012) reported the control sample without any GSE had *L. monocytogenes* and *E. coli* populations of 7.0 log CFU/g and 6.2 log CFU/g, respectively after 3 days. After the 15 day storage period, the control sample without any GSE was 8.4 log CFU/g for *L. monocytogenes* and 7.0 log CFU/g for *E. coli* (Shin and others 2012). This demonstrates that 1% GSE was not effective for reducing *L. monocytogenes* or *E. coli* populations on bacon.

Jang and others (2011) reported that GSE incorporated into rapeseed protein/gelatin films showed antimicrobial activity against gram positive and gram negative bacteria. A zone inhibition assay was utilized to measure the antimicrobial effect of 1.0 % GSE whereas the inhibition zones for *L. monocytogenes* and *E. coli* O157:H7 were 22.40 and 44.42 mm, respectfully (Jang and others 2011).

Grapefruit seed extract has some potential as a natural antimicrobial ingredient on beef homogenates, but not on bacon. Careful selection of GSE is needed to verify that the source of the extract has been extracted naturally. Although GSE has been studied using *in-vitro* and meat application models, further research is needed to evaluate its antimicrobial effects in RTE meat and poultry finished product applications.

Green Tea Extract

Green tea extract (GTE) has been noted for its antioxidant, anticancer and anti-inflammatory effects but it has most recently been investigated for its antimicrobial properties (Ferrazzano and others 2011). Green tea extract contains polyphenols, which are one of the most common and widespread groups of substances found in vegetative plant organs, flowers, and fruits (Kim and others 2004). The 10 most abundant volatile components of GTE — linalool, 6-cadinene, geraniol, nerolidol, a-terpineol, cis-jasmone, indole, P-ionone, 1-octanol, and caryophyllene — were examined for antimicrobial properties and found to have moderate activity so it could have potential as a broad spectrum antimicrobial (Kubo and others 1992). Commercial teas are usually classified into three major categories: unfermented containing catechins; fully fermented black tea containing catechins, theaflavins, and polymeric

thearubigins; and semi-fermented, usually black oolong, containing both catechins and theaflavins (Freidman 2007).

Juneja and others (2009) evaluated the effect of 3% GTE on thermal inactivation of *E. coli* O157:H7 in raw ground beef and found that the pathogen was more sensitive to the lethal effect of heat in the presence of GTE. Treatments with 3% GTE resulted in a 40.8% decrease in *D*-Value when cooked to an internal temperature of 55°C and 73.7% decrease in *D*-values at 60°C. The results indicate that GTE can decrease the heat resistance of *E. coli* O157:H7 (Juneja and others 2009). Juneja and others (2007) studied the antimicrobial effect of GTE on *C. perfringens* spore germination and outgrowth during extended cooling of cooked ground beef. Ground beef that was 93% lean combined with 1% GTE, containing 697 mg of total catechins per gram, was cooked in a 71°C water bath for 1 hour. The treatment with 1% GTE resulted in *C. perfringens* spore germination and out-growth populations of 4.29 log CFU/g compared to the control treatment without any GTE of 7.89 log CFU/g during an extended 15 hour chilling period (Juneja and others 2007). The study performed by Juneja and others (2007) is in agreement with findings by Sakanaka and others (2000) who reported that catechins from GTE reduced the heat-resistance of the spore-forming thermophilic spoilage bacteria *B. stearothermophilus* and *C. hermoaceticum*.

Kim and others (2004) conducted a study to evaluate the effectiveness of GTE to reduce the population of *L. monocytogenes* and *S. aureus* in raw ground beef stored at 7°C for 7 days. Viable cell populations of *L. monocytogenes* for treatments containing 0.1% (vol/wt) jasmine tea and 0.1% (vol/wt) GTE were 3.56 log CFU/g and 3.59 log CFU/g respectively. These

treatments were not different ($P \geq 0.05$) from the control treatment without any jasmine tea or GTE during the storage period with a population of 3.63 log CFU/g (Kim and others 2004). Additionally, viable cell counts of *S. aureus* treated with 0.1% (vol/wt) jasmine tea had a population of 4.54 log CFU/g and 0.1% (vol/wt) GTE had a population of 4.68 log CFU/g. These treatments were similar ($P \geq 0.05$) to the control treatment without any jasmine tea or GTE of 4.71 log CFU/g during the storage period (Kim and others 2004). Low antimicrobial activity of the GTE could have been due to the following factors: the 0.1% concentration of green or jasmine tea was not high enough to homogeneously disperse the GTE throughout the raw ground beef food matrix or proteins and fat from the ground beef may have protected the bacterial cells (Kim and others 2004). Furthermore, Kim and others (2004) reported the use of GTE in foods might be limited due to undesirable flavor in a product. Juneja and others (2007) were in agreement with Kim and others (2004) who stated that a concentration of 0.1% GTE was too low to show microbial inhibition in a food model system.

Green tea extract can inhibit spoilage bacteria as reported in a study by Lorenzo and others (2014) who found differences ($P \leq 0.05$) of total viable microbial populations between a treatment of self-made extract from green tea compared to a control treatment with no GTE in fresh ground pork patties containing 8.75% fat. In this study (Lorenzo and others 2014), the addition of 1 g/Kg of GTE increased the microbial shelf life of fresh ground pork patties packaged in 80% oxygen/20% carbon dioxide (CO₂) up to five days longer compared to the control treatment that contained 50 mg/Kg butylated- hydroxytoluene (BHT) when stored at 2 ± 1 °C. *Pseudomonas* bacteria are known to be responsible for the spoilage of aerobically stored fresh meat (Koutsoumanis and others 2006). Pseudomonads are gram negative bacteria that

are sensitive to CO₂ (Jay and others 2005), which is why most case-ready fresh meats are packaged in modified atmosphere packages that contain CO₂-enriched atmospheres (Lorenzo and Gómez 2012).

Lorenzo and others (2014) reported that the treatment containing GTE had lower ($P \leq 0.05$) populations of *Pseudomonas* spp. than the control treatment with no natural extracts in modified atmosphere packaged fresh pork patties over a 20 day storage period at 2 ± 1 °C. Additionally, GTE was evaluated against psychotropic bacteria that grow at refrigeration temperatures. By the end of 20 days of refrigerated storage, the fresh pork patties containing GTE had psychotropic bacteria counts of 5.6 log CFU/g compared to the control treatment without any natural extracts of 7.7 log CFU/g (Lorenzo and others 2014).

Green tea extract at a 0.1% concentration level dispersed homogeneously into a meat product can inhibit gram positive and certain gram negative bacteria (Lorenzo and others 2014). Depending on the extraction process that green tea undergoes, natural status for this extract is GRAS approved according to FDA. One potential concern associated with GTE could be strong undesirable flavors when used at concentrations needed in order to express antimicrobial effects as reported by Juneja and others (2007). Further research is needed to understand the efficacy of this natural extract in RTE meat and poultry applications.

Grape Seed Extract

Grape seed extract (GRSE) is generally recognized as safe (GRAS) as a food additive and known for antioxidant and antimicrobial properties in meat systems (Ahn and others 2004; Rababah and others 2004; Theivendran and others 2006). Silvan and others (2013) reported

the bioactive components of GRSE mainly consist of flavonols, phenolic acids, catechins, proanthocyanidins and anthocyanins. The composition reported by Silvan and others (2013) are consistent with previous studies on GRSE phenolic composition (Anastasiadi and others 2009; Rodríguez Montealegre and others 2006). Ahn and others (2002) reported 1% GRSE in ground beef retarded the formation of thiobarbituric acid–reactive substances (TBARS), hexanal, and warmed over flavor.

Ahn and others (2003) evaluated the antimicrobial effect of GRSE on *L. monocytogenes*, *E. coli* O157:H7 and *S. Typhimurium* populations monitored over a 9 day storage period at 4 °C in cooked lean ground beef. In this study by Ahn and other (2013), 1% GRSE was homogeneously mixed for 5 minutes into fresh ground beef and cooked to an internal temperature of 75°C in a convection oven. The cooked ground beef samples were cooled to room temperature and inoculated with approximately 5 log CFU/g *L. monocytogenes*, *S. Typhimurium* and *E. coli* O157:H7 (Ahn and other 2003). The cooked ground beef sample containing 1% GRSE resulted in *L. monocytogenes* populations of 6.49 CFU/g compared to the control sample without any GRSE of 6.90 CFU/g after 6 days at 4°C (Ahn and others 2003). Cooked ground beef samples containing 1% GRSE resulted in *E. coli* O157:H7 populations of 3.16 CFU/g compared to control sample without any GRSE of 4.25CFU/g after 6 days at 4°C. The cooked ground beef sample containing 1% GRSE resulted in *S. Typhimurium* populations of 3.47 log CFU/g compared to the control sample of 4.33 log CFU/g after 6 days at 4°C (Ahn and others 2003). Grape seed extract used in this study yielded a one to two reduction in gram negative pathogens such as *E. coli* O157:H7 and *Salmonella* Typhimurium and no practical effect against the gram positive pathogen, *L. monocytogenes* (Ahn and others 2003). The usage level of GRSE

(Ahn and others 2003) was higher than what was determined from a microbial susceptibility assay. This corresponds to previous research by Shelef and others (1984) who reported, in general, that the antimicrobial effect of natural extracts decreases in complex food systems.

In a study by Gadang and others (2008), nisin, malic acid, and GRSE were evaluated in whey protein isolate (WPI) films applied to turkey frankfurters inoculated with *L. monocytogenes* and then stored for 28 days at 4°C. Individual treatments of GRSE and nisin (1.0%, 2.0%, and 3.0%) incorporated into WPI films were not effective in inhibiting *L. monocytogenes*. However, 3.0% malic acid reduced *L. monocytogenes* populations by 2 logs CFU/g over the 28 day storage period. Gadang and others (2008) found the most effective treatment against *L. monocytogenes* was WPI films containing a combination nisin, malic acid and GRSE at 1.0% each, which reduced *L. monocytogenes* population by 5.0 logs CFU/g compared to the negative control that had no antimicrobial treatment. Gadang and others (2008) reported a synergistic effect between nisin, malic acid and GRSE thus resulting in increased antimicrobial efficacy against *L. monocytogenes* compared to when these individual components were used alone.

Grape seed extract is GRAS and could be considered a multifunctional ingredient, having antioxidant and some antimicrobial properties in meat applications. However, the use of high inclusion levels of GRSE, as reported by Gadang and others (2008), could lead to adverse organoleptic properties in a finished meat product. The use of multiple natural extracts or acidulants may aid in the overall effectiveness of an antimicrobial system, thus reducing the flavor impact. The practical use of GRSE in meat and poultry products may be limited due to

the low microbial reductions as reported by Ahn (2003) who showed less than one log reduction of *L. monocytogenes*, *E. coli* O157:H7 and *S. Typhimurium* resulted from the addition of GRSE in cooked lean ground beef after 6 days of storage at 4°C. Further research is needed to verify if GRSE incorporated into RTE meat and poultry formulations, as a food additive, has the same antimicrobial effects.

Plant Essential Oils

Plant essential oils (PEOs), also called volatile oils from plants, are aromatic oily liquids that contain terpenes, straight-chain compounds, benzene derivatives and miscellaneous compounds that are obtained from plant materials (Guenther 1948). They can be obtained by expression, fermentation, enfleurage or extraction but steam distillation is most the commonly used method for commercial production of PEOs (Van de Braak and Leijten 1994). Due to the extraction process that essential oils undergo, they contain volatile compounds that contribute higher amounts of aroma and flavor than plant extracts. Plant essential oils are permitted in food products as “flavorings” and considered GRAS by FDA in 21 CFR 182.20 (FDA 2015).

The inclusion level of PEOs to achieve antimicrobial effects in foods may be limited due to the strong flavor they impart to foods and their interaction with some food ingredients (Burt 2004). Pandit and Shelef (1994) reported 1% rosemary oil delayed *L. monocytogenes* growth in RTE pork liver sausage for 50 days stored at 5°C. Gill and others (2002) reported that 6% cilantro oil incorporated with a gelatin gel film inhibited *L. monocytogenes* growth in vacuum packaged cooked ham.

Rattanachaikunsopon and Phumkhachorn (2010) evaluated the antimicrobial activity of nine PEOs against *Salmonella* Enteritidis using the swab paper disc method. Based on the size of the inhibition zone (mm), basil oil had the highest antimicrobial activity against any of the various strains of *S. Enteritidis*. Rattanachaikunsopon and Phumkhachorn (2010) further tested basil oil added to Nham sausage inoculated with 5 log CFU/g of *S. Enteritidis* and stored at 4°C for 5 days. After three days of storage, Nham sausage containing 100 ppm basil oil resulted in an undetectable level of *S. Enteritidis* compared to a control treatment with no basil oil, which remained constant at 5 log CFU/g (Rattanachaikunsopon and Phumkhachorn 2010).

Singh and others (2003) also reported the antimicrobial potency of thyme oil reduced *L. monocytogenes* in beef hotdogs with a fat content of 0% and 9% fat but not in hotdogs containing 26% fat. Beef hotdogs with three fat levels (0%, 9% and 26%) were dipped into a 8 log CFU/g *L. monocytogenes* suspension for 2 minutes and air dried for 3 hours at 21°C to remove any excess moisture on the hotdog surface. The inoculated hotdogs were stored overnight in a refrigerator at 4°C to ensure sufficient bacteria attachment. The inoculated hotdogs were cut into 23-27 g samples using a sterile knife and placed into stomacher bags. A thyme oil and water suspension of 1.0 ml/L was added to the stomacher bags and hotdog samples were massaged for 5 minutes. The thyme oil suspension was drained from the bags and the hotdog samples were rinsed with sterile water to remove adhered thyme oil suspension. The hotdog samples were transferred to a sterile stomacher bag and massaged with 100 ml of sterile peptone for 2 minutes. The exudate was plated on PCA for *L. monocytogenes* enumeration. Beef hotdogs with 0% fat, treated with 1.0 ml/L thyme oil suspension for 5 minutes had a *L. monocytogenes* population count of 4.70 CFU/g compared to

a control without any thyme oil of 5.17 log CFU/g. Beef hotdogs with 9% fat, treated with 1.0 ml/L thyme oil suspension for 5 minutes had a *L. monocytogenes* population count of 4.81 CFU/g compared to a control without any thyme oil of 5.50 log CFU/g. Beef hotdogs with 26% fat did not have a significant *L. monocytogenes* population reduction compared to control samples. Beef hotdogs containing 26% fat, treated with 1.0 ml/L thyme oil suspension for 5 minutes had a *L. monocytogenes* population count of 5.49 CFU/g compared to a control without any thyme oil of 5.64 log CFU/g.

An important difference between PEOs and plant extracts is the hydrophobicity of PEOs. Plant essential oils are more soluble in the lipid phase than the aqueous phase of a food, which results in relatively less PEOs to act on the bacteria present in the aqueous phase (Mejlholm and Dalgaard 2002). Farbood and others (1976) suggested that a fat coat could form on the surface of bacterial cells and possibly prevent the penetration of the inhibitory substance of a spice into the cells. Furthermore, it is generally agreed that the high levels of fat and/or protein in foodstuffs protect bacteria from the action of the PEOs in some way (Pandit and Shelef 1994; Tassou and others 1995).

Plant essential oils have shown promising results *in vitro* and in various food models but further research is needed to understand their efficacy in RTE meat model systems. The mode of action for PEOs is unique compared to hydrophilic natural extracts. The insights learned from PEOs antimicrobial efficacy research by Rattanachaikunsopon and Phumkhachorn (2010), Pandit and Shelef (1994), Mejlholm and Dalgaard (2002), and Singh and others (2003) may be useful in development of natural antimicrobials for certain types of food products such as low-

fat meat products. Plant EOs in food model systems did not exhibit the same antimicrobial effect at the same *in vitro* assay concentration as witnessed by Pandit and Shelef (1994) who suggested that a 10-fold increase of rosemary oil was needed to control *L. monocytogenes* in pork liver sausage. It is also worth mentioning that not all PEOs from the same source have consistent antimicrobial efficacy. A number of variations have been reported in the chemical nature and the amount of volatiles of PEOs due to variations in the collection time of sample, abundance and/or lack of mineral components, distribution, changes in genetic levels, environmental conditions and the portion of the plant used for distillation (Salgueiro and others 1997; Venskutonis 1996). A summary of the reported reductions for natural multifunctional ingredients that may have potential application in meat and poultry products is shown in Table 4.1.

Table 4.1: Summary of reported reductions for natural multifunctional antimicrobial ingredients in meat and poultry products.

Ingredient	Substrate	Organism	Control Level	Reported Reduction	Conditions	Reference
Rosemary Extract						
	Cooked beef meatballs	Lactic acid bacteria	0.15% (w/w)	Significant, NR ^a	8°C for 6 days aerobic	Fernandez-Lopez 2005
	Chicken Meat Juice	<i>Campylobacter jejuni</i>	0.31 mg/ml	3 log	42°C for 24 hrs. 5% O ₂ , 10% CO ₂ , 85% N ₂	Piskernik and others 2011
	Chicken Meat Juice	<i>Campylobacter jejuni</i>	0.31 mg/ml	2.22 log	8°C for 120 hrs. 5% O ₂ , 10% CO ₂ , 85% N ₂	Piskernik and others 2011
	Fresh pork sausage (25% fat)	<i>Pseudomonas</i> & Lactic Acid Bacteria	260 ppm	Significant, NR ^a	4°C for 5 days Aerobic	Georgantelis and others 2007
	Fresh pork sausage (25% fat)	<i>Enterobacteriaceae</i>	260 ppm	Significant, NR ^a	4°C for 10 days Aerobic	Georgantelis and others 2007
Grapefruit Seed Extract						
	In-Vitro agar diffusion assay	<i>Listeria monocytogenes</i> MFBF	4.13% (m/V)	16mm	37°C for 18 hrs.	Cvetnić and Vladimir-Knežević (2004)
	In-Vitro agar diffusion assay	<i>Streptococcus faecalis</i> ATCC 20201	4.13% (m/V)	15mm	37°C for 18 hrs.	Cvetnić and Vladimir-Knežević (2004)
	In-Vitro agar diffusion assay	<i>Bacillus subtilis</i> NCTC 8236	8.25% (m/V)	14mm	37°C for 18 hrs.	Cvetnić and Vladimir-Knežević (2004)
	Moisture enhanced beef	<i>Escherichia coli</i> O157:H7	0.5%	Significant, NR ^a	0 hrs. Aerobic	Ko and others 2015
	Moisture enhanced beef	<i>Escherichia coli</i> O157:H7	0.5%	Significant, NR ^a	15°C for 48 hrs. Aerobic	Ko and others 2015
	Moisture enhanced beef	Aerobic plate counts	0.5%	Significant, NR ^a	15°C for 48 hrs. Aerobic	Ko and others 2015
	Chicken skin and meat model	<i>Campylobacter jejuni</i>	1.6%	3.05 log	1 min dip Aerobic	Riedel and others 2009
	Chicken skin and meat model	<i>Campylobacter jejuni</i>	1.6%	2.15 log	1 min dip + 24hrs at 5°C Aerobic	Riedel and others 2009
	Bacon wrapped with red algae	<i>Listeria monocytogenes</i>	1%	Significant, NR ^a	4°C for 6 days anaerobic	Shin and others 2012
Green Tea Extract						
	Cooked 90% lean ground beef	<i>Clostridium perfringens</i>	1% (697 mg catechins)	Significant, NR ^a	15 hr chill rate anaerobic	Juneja and others 2007
	Cooked 90% lean ground beef	<i>Clostridium perfringens</i>	2% (697 mg catechins)	Significant, NR ^a	15, 18, 21 hr chill rate anaerobic	Juneja and others 2007
	Cooked 75% lean ground beef	<i>Escherichia coli</i> O157:H7	3%	D-value: 40.8%	Int. Temperature 55°C anaerobic	Juneja and others 2009
	Cooked 75% lean ground beef	<i>Escherichia coli</i> O157:H7	3%	D-value: 73.7%	Int. Temperature 60°C anaerobic	Juneja and others 2009

Ingredient	Substrate	Organism	Control Level	Reported Reduction	Conditions	Reference
	Fresh ground beef	<i>Listeria monocytogenes</i>	0.1%	Not significant reduction	7°C for 7 days Aerobic	Kim and others 2004
	Fresh ground beef	<i>Staphylococcus aureus</i>	0.1%	Not significant reduction	7°C for 7 days Aerobic	Kim and others 2004
	Fresh ground beef	Total plate count	0.1%	Not significant reduction	7°C for 7 days Aerobic	Kim and others 2004
	Fresh pork patties	Total viable counts	0.1%	Significant, NR ^a	80% O ₂ /20% CO ₂ MAP 2°C for 20 days	Lorenzo and others 2014
	Fresh pork patties	Lactic acid bacteria	0.1%	Significant, NR ^a	80% O ₂ /20% CO ₂ MAP 2°C for 20 days	Lorenzo and others 2014
	Fresh pork patties	<i>Pseudomonas</i>	0.1%	Significant, NR ^a	80% O ₂ /20% CO ₂ MAP 2°C for 20 days	Lorenzo and others 2014
	Fresh pork patties	Psychotropic aerobic bacteria counts	0.1%	Significant, NR ^a	80% O ₂ /20% CO ₂ MAP 2°C for 20 days	Lorenzo and others 2014
Grape Seed Extract						
	Cooked lean ground beef	<i>Listeria monocytogenes</i>	1%	Significant, NR ^a	4°C for 9 days Aerobic	Ahn and others 2003
	Cooked lean ground beef	<i>Escherichia coli</i> O157:H7	1%	Significant, NR ^a	4°C for 9 days Aerobic	Ahn and others 2003
	Cooked lean ground beef	<i>Salmonella</i> Typhimurium	1%	Significant, NR ^a	4°C for 9 days Aerobic	Ahn and others 2003
	Turkey frankfurters; whey protein	<i>Listeria monocytogenes</i>	3%	Not significant reduction	4°C for 28 days Aerobic	Gadang and others 2008
	Turkey frankfurters; whey protein	<i>Listeria monocytogenes</i>	1% GRSE 1% malic acid 1%	Significant, NR ^a	4°C for 28 days Aerobic	Gadang and others 2008
Basil Essential Oil						
	Nham sausage	<i>Salmonella</i> Enteritidis	100 ppm	Significant, NR ^a	4°C for 5 days Aerobic	Rattanachai kunso pon and Phumkhachorn
Thyme Essential Oil						
	Beef Hotdogs Zero Fat (0%)	<i>Listeria monocytogenes</i>	1.0% (ml/l)	Significant, NR ^a	5 min. exposure Aerobic	Singh and others 2003
	Beef Hotdogs Low Fat (9%)	<i>Listeria monocytogenes</i>	1.0% (ml/l)	Significant, NR ^a	5 min. exposure Aerobic	Singh and others 2003
	Beef Hotdogs Full Fat (26%)	<i>Listeria monocytogenes</i>	1.0% (ml/l)	Not significant reduction	5 min. exposure Aerobic	Singh and others 2003

NR^a = significant reduction occurred, however reduction amount not reported

Chapter 5 - Conclusions

Current marketing trends indicate that some consumers desire 'natural' or 'clean-label' alternatives to synthetically derived preservatives in meat and poultry products. Meat and poultry manufacturers are challenged with understanding what can be incorporated into 'natural' meat and poultry products under the federal regulatory guidelines. As a result, many food manufacturers are reluctant to produce 'all-natural' products even though marketing data indicates this trend is increasing. Additionally, food safety is a priority for consumers when purchasing meat and poultry products.

Multifunctional ingredients are commonly used in meat and poultry products to solve the issue of labeling claims. Natural extracts and plant essential oils are GRAS approved by the FDA, which permits the use of these natural food additives in meat and poultry products pending they meet the criteria of minimally processed. Natural extracts and essential oils can add value to the meat product by exhibiting multiple functionalities and simplifying the finished product ingredient label.

Based on the reviewed scientific research, some natural extracts and plant essential oils have shown microbial inhibition against gram positive and gram negative bacteria using *in vitro* studies but there are concerns using these ingredients for increased food safety and shelf life extension. Grapefruit seed extract may be of particular interest to the meat and poultry industry as some research support that GSE can control gram positive and gram negative bacteria. Grapefruit seed extract has a potential as a broad spectrum antimicrobial using an *in*

vitro assay but further research is needed to determine if GSE is effective as an antimicrobial in meat and poultry applications.

Green tea extract at 1% was shown to be an effective antimicrobial treatment to control spore germination and outgrowth of *C. perfringens* during cooling of cooked ground beef. A usage level of 1% GTE however, may impart undesirable flavors in the finished product, which would limit its use in meat and poultry products. Green tea extract could contribute an undesirable flavor and be too expensive for an antimicrobial treatment in meat and poultry products.

In processed meat and poultry products that contain seasonings, basil oil may have merit as an effective antimicrobial to control pathogens and extend product shelf life. Additional research is needed to investigate basil oil in additional meat and poultry applications. The application for thyme oil as an antimicrobial dip treatment is not practical to the meat and poultry industry. Further research is needed to investigate whether thyme oil added directly into meat and poultry products is efficacious as an antimicrobial treatment.

Previous research has shown that natural extracts and plant essential oils exhibit antimicrobial properties. Grapefruit seed extract, grape seed extract, rosemary extract, green tea extract and basil oil have all shown microbial inhibition *in vitro* while incorporating these ingredients into RTE meat and poultry products has yielded inconsistent results. In food model systems, an increased concentration of natural extracts and essential oils was needed to produce similar results as observed using *in vitro* experiments. Adding to the complexity, since natural extracts and essential oils are harvested from natural sources, product variation within plant species is expected. Ingredient suppliers must establish certificate of analysis

requirements on natural extracts and essential oils to ensure active components are consistent. Multifunctional ingredients that meet the regulatory approval for natural products such as natural extracts and essential oils may be suitable candidates for effective antimicrobials for natural and clean-label meat and poultry products but further research is needed to confirm the antimicrobial efficacy in specific meat and poultry applications.

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