THE EFFECT OF GRAPE POMACE EXTRACT AS AN ANTIOXIDANT IN GOAT MEAT SAUSAGE

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THE EFFECT OF GRAPE POMACE EXTRACT AS AN

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This study compared Cynthiana grape pomace extract (GPE) and commercial grape seed extract (GSE) to common antioxidants used to prevent lipid oxidation in fresh goat meat sausage. Sausages were manufactured from lean goat meat (70 %), goat fat (30 %) and 2.7 % smoked polish kielbasa seasoning spices. Antioxidants GPE, GSE, and butylated hydroxytoluene (BHT) were added to the sausage at levels of 1.135, 5.625, and 3.405 g/5.0 kg sausage (respectively) to test their effectiveness. Fresh sausage was fabricated, placed on foam trays, overwrapped with PVC, then refrigerated at 4 C° for 28 days. The total antioxidant activity of (GSE) and (GPE) was measured in this study using the oxygen radical absorbance capacity (ORAC) method. The results showed that the (GSE) had a higher ORAC activity than the (GPE). Also, the effect of these natural antioxidants on the quality of goat sausages microbial content, lipid oxidation, color, sensory attributes, and proximate analysis was determined. The L* value of all the samples increased. However, the* redness and b* yellowness decreased ($p<0.05$) during storage. The GSE-containing samples showed slightly lower thiobarbituric acid formation of TBARS, followed by GPE, after 28 days. GSE and GPE did not significantly decrease ($p<0.05$) microbial growth compared to the controls. The antimicrobial effect of GSE and GPE did not help to prevent microbial spoilage in goat meat sausages.

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CHAPTER I

INTRODUCTION

General information

Meat is a perishable food item, and meat spoilage significantly impacts the meat industry. The spoilage of meat and meat products is highly correlated with shelf-life (Kusuma 2008). Appearance, texture, and flavor are very important sensory properties and that most consumers use to judge meat quality by consumers. Meat color is considered as the first limiting issue in shelf-life because customers tend to use meat color as the primary indication of freshness (Kusuma 2008). So, visual appearance is the most significant property of any meat product because it strongly influences the consumers purchase choice (Lopez-Bote and others 1998).

Consumers choose bright-red meat products. Due to the presence of oxygen, the fresh looking color of meat is usually short-lived (Murcia and Martinez-Tome 2001). Many factors can be responsible for discoloration but the consumer will usually relate this to bacteria growth which is not always the reason (Seideman and others 1984). Many programs and agencies are making food safety and quality a high priority.

For example, The Robert M. Kerr Food & Agricultural Products Centre (FAPC) Global Food Safety Initiative (GFSI) program is providing services to meet both safety food and security needs of Oklahoma's food industry. Globalization of the food industry effects on almost every Oklahoma food processor, both directly and indirectly. For instance, The FAPC-GFSI program focuses on direct food industry assistance in different areas such as training, reviewing, pre-third-party audit preparations, education, and in-plant technical assistance for food safety and quality programs. The Global Food Safety Initiative is a business-driven initiative for the continuous improvement of food safety management systems to ensure confidence in the delivery of safe food to consumers worldwide.

There are two major reasons why meat products naturally spoil. The first reason is microbial growth and the second reason is chemical deterioration. The most common form of chemical deterioration is oxidative rancidity known as lipid oxidation (Sebranek and others 2005). Lipid oxidation is the complex interaction of lipids with oxygen which leads to decomposition of triacylglycerols and phospholipids and leads to production of small volatile molecule (off- aromas) known as oxidative rancidity. These volatile compounds help to deteriorate food and the loss of nutritional quality of the food (McCements and Decker 2008).

Meats are susceptible to lipid oxidation which effect characteristics such as flavor, color, texture, nutritive value, and subsequently limits their shelf-life (Kanner 1994). This leads to loss of meat freshness that discourages repeat purchases by consumers and that also leads to results in consumer dissatisfaction (Sebranek and others 2005). Therefore, the meat industry is faced with the challenge of developing strategies for preventing lipid

oxidation in meat foods. The use of either synthetic or natural antioxidants is one of the main strategies for avoiding lipid oxidation in food.

The most effective antioxidants hold aromatic or phenolic rings capable of donating a hydrogen atom (H•) to the free radical formed during lipid oxidation. The use of synthetic phenolic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) is a very effective way to prevent the warmed over flavor (WOF) for occurring. However, consumer concern regarding the safety of these synthetics has encouraged food manufacturers to investigate the benefits of natural antioxidants as replacements. Various plant materials containing polyphenolic compounds are effective antioxidants and can retard the development of WOF in meat systems (Kulkarni and others 2011). Many researchers have found that selected plant extracts are efficient at reducing oxidative changes during storage and meat processing. However, little information is available in the literature on antioxidative effects and physicochemical changes when grape pomace extract is added as a natural antioxidant to muscle meats (especially goat).

Goat meat is considered to be "the most widely-consumed meat in the world" (Alford March 31, 2009). Also, goat meat is available in high quantities in developing countries, and represents 94 percent of the world total (Babiker and others 1990). Goat meat like other red meats has unsaturated fatty acid which can easily get spoil by lipid oxidation. Meat sausage is a good example for processing meat in order to increase the shelf life and avoid oxidative rancidity in products.

It is the aim of this study to provide some of the required information for using antioxidant in fresh goat- meat sausages, and specific study objectives are:

1- Measure the antioxidant capacity of the following natural antioxidants from grape by oxygen radical absorbance capacity (ORAC):

   a. Extract from Oklahoma Cynthiana (Vitis Vinifera) grape pomace.

   b. Commercial grape seed extract (Nusci Walnut, CA 91788, USA), containing (95 percent proanthocyanidins standardized extract powder and distributed by: www. Herb store USA.com.

2- Perform sensory analysis of goat meat sausage with and without antioxidants: grape seed extract, grape pomace extract, and synthetic antioxidant at the beginning of the study (day 0) and after 28 days of refrigerated storage. Sensory analysis will include:

   - Flavor
   - Off-flavor
   - Color

3- Analyze goat meat sausage samples at the beginning and end of the shelf life study for protein, fat, ash, and moisture.

4- Analyze sample color change during the shelf life study at days 0 and 28 using a Chroma meter.

5- Perform a microbiological total plate count analysis on samples at days 0 and 28 of the shelf-life study.

6- Determine lipid oxidation by using 2-Thiobarbiyuric acid reactive substances (TBARS) test at day 0 and every 7^{th} day of the shelf life study.

7- Compare the goat meat sausage samples with antioxidants added (commercial grape seed extract, grape pomace extract, and synthetic antioxidant) to the control to determine the effects of the antioxidant related to shelf-life.

CHAPTER II

REVIEW OF LITERATURE

Antioxidant

Antioxidant activity is defined as the ability to reduce free radical formation and scavenge reactive oxygen species (Reyes-Carmona and others 2006). Plant phenolic is a good natural antioxidant example which has diverse group of phenolic compounds such as flavonoids, and anthocyanins. Many antioxidants slow lipid oxidation by scavenging free radicals by reacting faster with free radicals than unsaturated fatty acid. So, antioxidant efficiency is dependent on the ability of free radical scavenger (FRS) to donate hydrogen to a free radical.

Natural antioxidants molecules

The free radical is an atom or compound which has an unpaired electron such as a hydrogen atom. Free radicals are produced in abundance in all cells. But, there are numerous natural defenses to prevent their formation or to neutralize them after they are produced. Some examples of natural antioxidant are listed below in no particular order:

1. Antioxidant enzymes such as: Catalase, glutathione, glutathione reductase, superoxide dismutase.
2. Metal binding proteins such as hemoglobin, myoglobin, ferritin and metallotheinein
3. Common antioxidants (radical scavenger) like vitamin C, E, carotenoids (β-caroten, lycopene, etc.), thiols (R-SH).
4. Other antioxidants include metals such as copper, zinc (as CUZn-SOD), selenium (GPx), manganese (MnSOD), reduced glutathione (GSH).

   (Knight 1998)

Synthetic antioxidants

Synthetic phenolic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroxyquinone (TBHQ) are the most common synthetic antioxidants. they are used as food additives to increase the shelf life for food products (Baydar and others 2007) by inhibiting warmed over flavor (WOF) (Kulkarni and others 2011), preventing oxidation in fresh meats (Garrido and others 2011), and reducing lipid oxidation (Rababah and others 2011).

Recently, it has been realized that these products may have toxic, carcinogenic effects on humans and abnormal effects on enzyme systems (Baydar and others 2007). Hence, researchers have recognized the need to identify new natural antioxidants to use as safe additives in the food industry (Gamez-Meza and others 2009). Numerous plant materials containing polyphenolic compounds are effective antioxidants and can reduce WOF in meat. For instance, grape seed extract can reduce lipid oxidation and WOF in

cooked ground beef when added at between 0.1 percent and 1percent (Ahn and others 2007).

The interest in natural antioxidants, especially of plant origin, has greatly increased in recent years. Natural antioxidants can help to protect the human body from chronic diseases including cancer, cardiovascular diseases, and cataract. The antioxidant properties of plant extracts have been recognized to contain polyphenols. Plants which have a high-level of polyphenols have great natural antioxidant capacity. Grape skins, seeds, and stems, waste products generated during wine and grape juice processing, are rich sources of polyphenols as reported by Baydar and others (2007).

History of antioxidants

Oxygen toxicity was initially first described in1878 but was not established until 1899. The first experiment related to a free radical was conducted by Fento in 1894 as reported by Knight (1998).The history of antioxidants started in 19th century when it was discovered that the deterioration of natural rubber was caused by peroxidation, not by biological processes as had previously thought. In the late 19th century, the researchers found they could add specific chemicals to improve the rubber to prevent oxidation. Studies in the 1950s showed that biological antioxidant helped to prevent some diseases (Scott 1997). After, 1960s, scientists started to study the effect of free radicals in chemistry, polymer, and food science research. This research has expanded and now includes biological and medicinal applications (Kanner 1994).

Antioxidants from grapes

Grapes are one of the largest fruit crops in the world. grapes are used for wine production, Leaving about 10 million tons of waste (pomace) annually, after the juice is extracted as reported by Kammerer and others (2004).

Many studies have shown that grapes are used for medical purposes (Stevenson and Hurst 2007); (Meyer and others 1997), animal feed (Goñi and others 2007), cosmetics (Baumann and others 2009), and to increasing shelf life for meat and meat products (Kulkarni and others 2011).

Grapes have a high amount of procyanidins which are the main class of polyphenols. They have astringent and bitter tastes (Le Bourvellec and others 2005). Polyphenols are considered as the third highest component in grapes and wines after carbohydrates and fruit acids. Murthy and others (2002) reported that polyphenol compounds in grape are (+)-catechin (11 percent), epicatechin- (4âf8)-epicatechin (dimer B2) (6percent), (-)-epicatechin (10percent), epicatechin 3-O-gallate-(4âf8)-catechin (B1-3-O-gallate) (7 percent), and (-)-epicatechin 3-O-gallate (9 percent) which have a variety of health benefits. Nawaz and others (2006) have reported that polyphenol compounds have various classes which ranged from phenolic acids, colored anthocyanins, simple flavonoid and complex flavonoids. Flavonoids are reported by Carpenter and others (2007) as the most abundant and potent group of plant phenolic compounds which act as antioxidants.

Antioxidant from grape pomace

Grape pomace is around 20 percent of the weight of the grape processing (Sáyago-Ayerdi and others 2009). Grape pomace has a variety of benefits including anti-

microbial (Baydar and others 2004), (Özkan and others 2004), anti-oxidants (Ahn and others 2007), anti-ulcer (Saito and others 1998), anti-mutagenic, anti-carcinogenic (Nawaz and others 2006; Lau and King 2003); (Bonilla and others 1999), and anti-inflammatory activities (Perumalla and Hettiarachchy 2011).

The benefits are the result of the composition of antioxidant compounds (polyphenols), which absorb free radicals from the body. Polyphenols are reported by Macheix and Fleuriet (1990) as the greatest abundant secondary metabolites found in plants and are roughly classified in the plant kingdom. Polyphenols can be classified according to their chemical structure and activity. Besides, bioactive compounds appear to have positive health effects (Garrido and others 2011).

The amount of total extractable polyphenol found in fresh grape tissue is around 10 percent in the pulp; 60-70 percent in the seed; and 28-35 percent in the skin (Nawaz and others 2006). Grape skins and seeds have a high amount of flavonoids including monomeric phenolic compounds, such as (+) catechins, (−) epicatechin, and (−)-epicatechin-3-O-gallate and dimeric, trimeric, and tetrameric procyanidins. Studies have shown that flavonoids act as powerful antioxidants by scavenging free radicals and inhibiting oxidative rancidities (Brenes and others 2008).Table (1) shows the Basic composition of grape pomace concentrate (Goñi and others 2007).

Table 1, Basic composition of grape pomace concentrate.

Item	Dry matter (g/kg)
Protein	138.5 ± 0 1.20
Soluble sugars	20.70± 0.30
Fat	9.87 ±0.17
Fiber	151.80 ±0.72
Ash	24.10 ±0.30
Extractable polyphenols	48.70 ±0.07
Hydrolyzable polyphenols	26.60 ±0.05
Condensed tannins	150.90 ±0.05

Antioxidant from grape skin

Grape skins are around 82 percent of the wet weight of wine grape pomace. Grape skins contain various types of polyphenols, including 39 types of anthocyanins, hydroxycinnamic acids, catechins, and flavonols as reported by Deng and others (2011), Grape skin has a high amount of anthocyanidins and anthocyanins, natural pigments with antimutagenic activities and antioxidant activity (Rockenbach and others 2011).

Antioxidant from Grape seeds

Grape seeds have around (w/w) 40 percent fiber, 16 percent triglyceride oil, 11 percent protein, 7 percent complex phenolic compounds like tannins, sugars, minerals, and other substances (de Campos and others 2008). Grape seed can be separated, extracted, dried and purified into grape seed extract (GSE) which contains phenolic compounds. Therefore, GSE has been evaluated for its antioxidant activity (Lau and King 2003). Brannan (2008) reported that the polyphenolics in GSE are mainly condensed tannins, a.k.a. proanthocyanidins, usually oligomers and polymers of polyhydroxy flavan-3-ols, many in the form of gallate esters or glycosides. Also, procyanidin dimers, trimers, and highly polymerised procyanidins and gallic acid are significant components in grape seeds (Chedea and others 2010); (Adámez and others 2012)

l (GSO) has many excellent nutritional properties such are: cholesterol free, low in saturated fats, contains linoleic acid and high-density lipoprotein, and rich in Vitamin E

and (GSO) oil has a high smoke point, of 252 C^{o}; therefore, (GSO) is a good choice for frying and other high temperature food applications (Roberts and others 2008).

Extraction methods

There are several methods used to get grape seed extract. Some extraction methods were done with solid phase extraction (SPE) (Chedea and others 2010), and some extraction methods use organic solvents. These extraction procedures were effective, but the extracts were harmful for humans ingesting because of toxic residual solvent.

In the past, solvents, such as hexane and methanol combinations (Santos-Buelga and others 1995), ethyl acetate (Mandic and others 2009); (Bonilla and others 1999), ethanol–benzene combinations (Kofujita and others 1999), acetone and water combination (Pekić and others 1998), and sulfur dioxide have been used (Cacace and Mazza 2002). All these solvents are very dangerous and toxic to humans when digested in large amounts. On the other hand, Nawaz and others (2006); Gamez-Meza and others (2009) showed that the extraction with ethanol and water is a safe and efficient method to extract polyphenol compounds.

Laboratory evaluation methods:

Antioxidant capacity

There are several methods used to determine antioxidant capacity such as total radical absorption potentials (TRAP), DPPH (2,2- diphenyl-1-picrylhydrazyl), ABTS (2,2′-azinobis (3 ethylbenzothiazoline 6-sulfonate)), ferric reducing antioxidant potential (FRAP), superoxide dismutase (SOD), and ORAC (Dudonné and others 2009).

Oxygen radical absorbance capacity (ORAC) is considered as a standard tool to measure antioxidant activity in the pharmaceutical, nutraceutical, and food industries (Huang and others 2002). Also, ORAC assay is the most appropriate method to measure the total antioxidant capacity of foods such as fruit and vegetables because it accounts for both inhibition time and degree of inhibition of free radical action as reported by Dansby (2006). ORAC values in fruits and leaves of blackberries, raspberries, and strawberries are influenced by cultivars, maturity stages, and phenolic, anthocyanin content (Wang and Lin 2000).

The ORAC assay measures antioxidant scavenging activity against peroxyl radicals produced by 2,2 '-Azobis(2-Amidinopropane) hydrochloride (AAPH). This antioxidant activity is evaluated by comparing the test sample area under the fluorescence decay curve (AUC), 6-hydroxy-2 5 7 8-tetramethylchroman-2-carboxylic acid (trolox), and a blank sample which has no antioxidant (Scott 2012).

Sensory evaluation

Sensory evaluation is defined as "a scientific discipline used to evoke, measure, analyze, and interpret reactions to those characteristics of food and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing (Stone and others 2012). Odor and flavor are good examples of sensory evaluation which help to show the effect of antioxidant on the product. There are many studies in food and meat sciences that have found that there is a correlation between adding antioxidants and increased shelf life and improved sensory attributes in muscle food.

A descriptive panel (Brannan, 2009) was used to measure the odor and flavor and the result was compared the control with antioxidant treatment on muscle food. showed that GSE was effective in limiting the intensity of musty and rancid odor, two attributes that are commonly associated with WOF, in precooked ground chicken breast after 12 days of refrigerated storage. These results are in agreement with Kulkarni and others (2011) who reported that rancid odor and grassy odor were reduced by GSE at concentrations of 300 ppm and 500 ppm in beef sausages after 4 months of frozen storage. Moreover, Rojas and Brewer (2007) reported that rancid and wet-cardboard odors were decreased by GSE in cooked beef and pork after storage at 4 °C for 8 days.

For rancid and spoilage flavors Brannan (2009) reported that 0.1% GSE (w/w) was an effective antioxidant which reduced attributes associated with WOF (such as rancid flavor) compared to control in precooked ground chicken breast after 12 d of refrigerated storage. In contrast Carpenter and others (2007) reported that addition of GSE (400 and 1000 micro-g/g) and bearberry (BB) (80 and 1000 micro-g/g) did not adversely affect the sensorial properties of cooked pork after 4 d storage at 4 °C.

Proximate analysis

Fat, protein, ash and moisture are important analyses performed on food products. There are several methods used to determine proximate analysis recommended by AOAC. Moisture content is considered as a one of the most necessary and important analytical procedures which can be performed on food products. There are three forms of water in foods (free water, adsorbed water, and water of hydration). Depending on these

forms of water present in a food, the method used for evaluating moisture may measure high or low moisture present (Robert and Bradley 2010).

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in food product. There are two types of ashing: wet ashing and dry ashing. Ashing is part of the proximate analysis and it is the first step in order to prepare samples for certain types of elemental analysis (Marshal 2010).

Lipids are a group of substances that, in general, are soluble in some organic solvent but are sparingly soluble in water. The total lipid content of a food is commonly determined by organic solvent extraction methods or alkaline or acid hydrolysis. The Sohxlet method is a good example for organic solvent extract (petroleum ether).

Protein is an abundant compound in all cells, and most of proteins are important for biological functions and cell structures. Many methods have been developed to measure protein content. Determination of nitrogen, peptide bonds, aromatic amino acids dye- binding capacity, and ultraviolet absorptivity of protein are the basic principles of these methods. The principle of the Kjeldal method is a good example for measuring nitrogen in food to determine the amount of protein percent in food. Since proteins have high basic amino acids which contain more nitrogen (Chang 2010).

Color analysis

The color of fresh meat has a significant impact to an every aspect of the meat industry. Color changes and color intensities should be understood in order to reduce off-colors which may eventually appear in the food products (Seideman and others 1984). Color is a necessary visual sign involved in consumer perception of meat quality (Carpenter and others 2007). Consumers equate the color of meat to freshness and quality

(Seideman and others 1984). Different (L*), values which represent lightness and (a*), and (b*) values representing redness and yellowness, respectively were measured to determine the effect of antioxidant on meat color. Many studies have been conducted on color change and some examples follow:

Garrido and others (2011) showed that the L* value of pork burgers decreased (darker meat) on day 6 of a shelf life study at 4^{o}C when grape pomace extract (0.06 g/100 g finale product) was added. The result agrees with pervious research. For example, Brannan (2009) reported that the GSE in ground chicken breast (0.1 percent) caused significantly darker (L*) color after 12 days at 4^{o}C. Also, Ahn and others (2007) found that addition of 1.0 percent GSE (ActiVinTM) and 1.0 percent pine bark extract (pycnogenols) decreased the lightness (L*) values of cooked beef after 9 days of refrigerated storage.

In contrast, others researches Carpenter and others (2007) did not find any significant differences in L* values of raw pork patties after 12 days at 4^{o}C of storage when the concentration of both GSE and BB were increased to (0–1000 µg/g muscle). On the other hand, Kulkarni and others (2011) showed L* value of all beef sausages increased over time however, after 3 months of frozen storage, L* values of samples containing 100 ppm GSE were higher than samples containing 500 ppm GSE, ascorbic acid (AA, 100 ppm of fat) and propyl gallate (PG, 100 ppm of fat) during storage

Rojas and Brewer (2007) claimed that the color values of refrigerated cooked beef and pork patties were unaffected by the antioxidant treatment (GSE concentration) for 8 days. In the same study a* values, were not affected by addition GSE. On the other hand, a study by Carpenter and others (2007) reported that the a* redness values in raw pork

patties decreased over the 12-day storage period. Addition of GSE resulted in minor increases in a* redness values of raw pork patties, relative to controls, after 6, 9 and 12 days of refrigerated storage at 4^{o}C. Brannan (2009) showed the concentration of GSE used in ground chicken breast (0.1 percent) caused significantly redder color (a*). This agrees with previous research of Ahn and others (2007) which found the addition of ActiVinTM significantly increased the redness (a*) value. However, the addition of pycnogenols prevented a decrease in a* values when compared to the control. In addition, Rababah and others (2011) showed that the addition of GSE significantly ($p < 0.05$) increased the redness in goat meat at 5◦C after 9 days of storage.

Brannan (2009) reported GSE added to ground chicken breast (0.1 percent) caused significantly less yellowness (b*) in patties. However, Ahn and others (2007) showed no significant differences in yellowness (b*) values for the control and treatment with BHA/ BHT and Herbaloxs added in to refrigerated storage study.

Also, Carpenter and others (2007) showed that GSE and BB addition did not change b* yellowness values for raw pork patties and no obvious data trends were observed. In contrast, beef samples containing water-soluble oregano extract had higher b* values compared to samples containing GSE and RE (Rojas and Brewer 2007).

Microbiological

Meat safety issues are often very serious and may result in health problems for consumers. Recalls of potentially contaminated products may be related to microorganisms, especially bacterial pathogens (Sofos 2008). “In recent years, some highly publicized outbreaks of foodborne disease in the United States, caused by

pathogenic bacteria such as Escherichia coli O157:H7 and Listeria monocytogenes, have brought meat safety and associated issues to the forefront of societal concerns" as reported by (Sofos 2008).

The effect of natural antimicrobials and antioxidants in meat products are in agreement with a number of studies. As far as the antimicrobial effects of GSE ActiVin TM and pine bark extract (pycnogenols), at 1.0 percent levels are concerned, numbers of E. coli O157:H7 in the first 3 days of refrigerated storage were rapidly reduced (Ahn and others 2007). In order to increase the shelf life of low sulphite beef patties, GSE would be mainly effective against gram positive bacteria, with gallic acid as the main active component, and green tea extract (GTE) would inhibit E. coli, S. aureus, Staphylococcus epidermidis and Streptococcus mutans (Banon and others 2007).

Georgantelis and others (2007) reported that samples containing chitosan generally had lower microbial counts compared to rosemary alone, α tocopherol alone or the control. The antimicrobial activity of chitosan is documented both in vitro and in situ against a number of food spoilage and pathogenic microorganisms, including among others Staphylococcus aureus, Pseudomonas aeruginosa, Shigella dysenteriae, Bacillus cereus, Proteus vulgaris, Escherichia coli, Vibrio spp. and Salmonella typhimurium and several yeasts and molds. Fresh pork sausages were treated with 0.5 percent and 1 percent chitosan. Both treatments increased the shelf-life of sausages stored at chilled temperatures for 28 days. However, the samples without chitosan had already exceed the maximum levels acceptable after 14 days because, chitosan has a high antimicrobial activity at a level of 1 percent and high antioxidant effects at level of 0.5 percent (Soultos and others 2008).

This is in contrast to previous reports where the addition of red grape pomace extracts did not affect the spoilage of burger packed under aerobic conditions because the antioxidants did not completely prevent spoilage during storage (Garrido and others 2011). Also, the addition of GSE and BB did not significantly affect or improve the microbial status of pork patties relative to the controls (Carpenter and others 2007).

Measurement of Lipid oxidation

German (1999) defined "Lipid oxidation as a multi-step, multifactorial process, and the variables encompassed in foods include individual fatty acid susceptibility, molecular structure of lipids, physical state of lipids, initiation reaction, hydroxide decomposition catalysis (metals), presence of oxidized lipids, and the amounts and selection of antioxidant present".

Oxidative decomposition is an important issue for both satisfactoriness and nutritional food product quality. Different tastes, sensory evaluation, physical, and chemical methods have been developed to measure lipid oxidation in foods. However, a particular method cannot help to evaluate all the possible oxidative reactions that may occur during lipid oxidation because of the multitude of fats in unique foods under different conditions (Monroy and Cecilia 2007).

There are several examples of methods that can be used to measure proxides and related products: conjugated diene hydroperoxides (CD) is used to measure higher polyunsaturated fatty acid. Carbonyl value is used to measure a variety of secondary products; volatile analyses (VA) is used to measure volatile compounds that are produced from lipid oxidation; the 2-thiobarbiyuric acid reactive substances (TBARS) value is used to evaluate the extent of lipid oxidation in foods; and peroxide value (PV) is used to

measure simple peroxides (the primary products of lipid oxidation) (Monroy and Cecilia 2007).

(TBARS) value is the most common method used to evaluate the extent of lipid oxidation in foods (Monoroy 2007). TBARS is used to measure lipid oxidation in muscle meat (Carpenter and others 2007; Ahn and others 2006; and Kulkarni and others 2010). This method is based on spectrophotometric quantitation of the red colored complex formed after the reaction of malonaldehyde (MDA) with 2-thiobarbiyuric acid (TBA) and secondary products derived from lipid oxidation (Monroy and Cecilia 2007).

Antioxidant in food products

Food products which contain oil or fat are the most food influenced by lipid oxidation during storage. Undesirable changes such as color change, loss of nutrients are caused by lipid oxidation reactions in muscle foods (Brannan and Decker 2001). As the fat decomposes and reacts with oxygen, peroxides are produced. Peroxides give a rancid smell and soapy flavor to a rancid food. Antioxidants prevent the formation of peroxides. Adding an antioxidant such as vitamin E to vegetable oil is a good example of preventing the formation of peroxides in a food product (Food Additives and Ingredients Association and Chemical Industry Education Centre 2008).

There are several food products which have oil or fat in their ingredients or physical makeup such as snacks, cakes, meat, fish, poultry, margarine, dairy products, and potato products. Antioxidants are added to the food products which contain unsaturated fats (fatty acids which have double bonds in their aliphatic chain) to make them last longer and prevent them from turning rancid (Food Additives and Ingredients Association and Chemical Industry Education Centre 2008). Rababah and others (2004)

found that active antioxidant compounds in grape seed and green tea extracts were greater than several other plants such as fenugreek, black tea, ginger, and rosemary. Grape seed has a high amount of bioactive compounds. One study found that grape seed's antioxidant potential was 20 and 50 fold greater than vitamins E and C, respectively (Carpenter and others 2007).

Antioxidant in meat

Intake of meat containing high amounts of polyunsaturated fatty acids has increased significantly in the last ten years. This behavior has led to the nutritionists' recommendations to decrease the intake of polyunsaturated fatty acids. The high degree of polyunsaturation can accelerate the oxidative processes and cause meat to lose its color, texture, flavor, and nutritional value (Mielnik and others 2006).

Adding antioxidants is the most common strategy for preventing lipid oxidation in foods (Sáyago-Ayerdi and others 2009). Several authors mention the effect of natural antioxidant in muscle meat (pork: (Carpenter and others 2007; McCarthy and others 2001; Garrido and others 2011); beef: (Rojas and Brewer 2007; Ahn and others 2006; Ahn and others 2007; Banon and others 2007), poultries: (Brannan 2008; Mielnik and others 2006; Lau and King 2003), fish muscle: (Pazos and others 2005); and goat meat: (Rababah and others 2011).

There are many studies which have shown that natural antioxidants reduce lipid oxidation in meat and meat products. Significant interest has recently been focused on the addition of natural antioxidants to foods to replace synthetic antioxidants, due to their potential to prolong the shelf life of food products by inhibiting and delaying lipid

oxidation (Sánchez-Alonso and others 2008). Antioxidants have been documented to minimize lipid oxidation in processed food at levels reported safe for human consumption (Rababah and others 2011).

Antioxidants and plant extracts have been reported to reduce the TBARS values in raw meats during storage (Ahn and others 2006). GSE has been evaluated for its antioxidative effect on a few meat types and has been reported to improve the oxidative stability of goat meat (Rababah and others 2011), turkey patties, and cooled stored turkey meat (Lau and King 2003; Mielnik and others 2006).

CHAPTER III

MATERIALS AND METHODS

Plant materials and chemicals

Grapes (Cynthiana) were provided for this research from a research station center in Oklahoma State University. These following steps were used to process grape pomace. Wine was made and pomace was collected for an extraction procedure (described below) that would result in an antioxidant compound known as (GPE). The entire amount of grape pomace was separated from the wine and placed in liquid nitrogen (-196° C) using a metal strainer. Frozen grape pomace was grounded in a Waring blender jar (51BL31) and ground for 30 seconds in a 4° C room. The resultant powder was placed in vacuum bags (20.32 x 25.4 cm) vacuum pouches, Mid-Western Research and Supply, Inc) and stored at -20° C for later use and analysis.

Water and alcohol were also used in the extraction process. Ethyl alcohol, of analytical reagent grade, was purchased from Fisher Scientific (Fair Lawn, NJ). Water was processed by a Milli-Q purification system Millipore, Bedford, MA, USA).

Extraction procedure

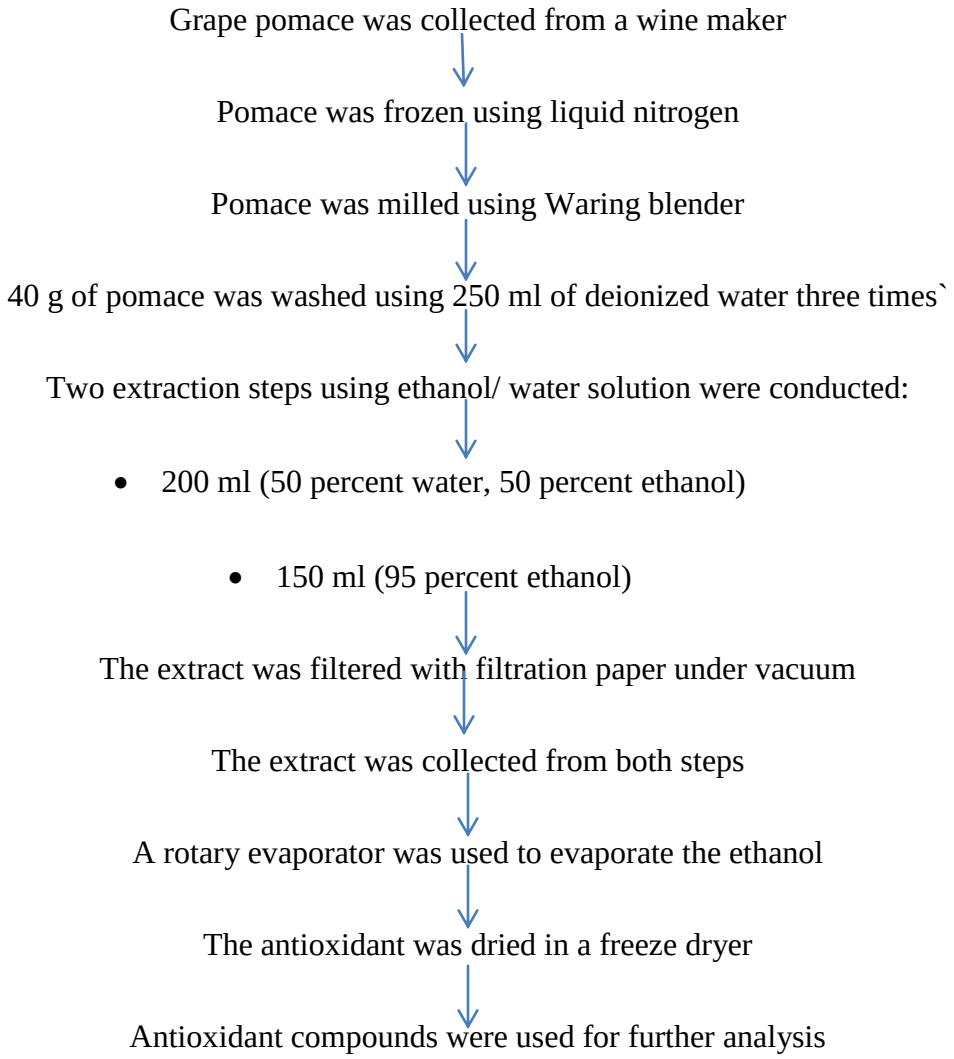
Figure 1 shows the extraction procedure for grape pomace. The extraction was carried out according to the method described by (Nawaz and others 2006). Around 40 g of milled grape pomace was mixed with a 200 ml of a 50 percent ethanol/ water solution. This was then mixed for 5 minutes using a magnetic stirrer and allowed to extract in the dark for 1 hour. The top phase portion was filtered under vacuum through Whatman filter paper #4 (9.0 cm diameter) to residue solid.

The residue was mixed with 150 ml of 95 percent ethanol/ water solution for 5 minutes and then allowed to extract in the dark for 1 hour. The top phase was decanted and filtered again under vacuum through Whatman filter paper #4 (9.0 cm diameter). The extract from both steps was collected.

A rotary evaporator (11590, Brinkman,Cantagne Road, Westbury, NY) was used to evaporate the ethanol from the extract. A freeze dryer (64132, LABCONCO, Kansas, Missouri) was used to evaporate the water from the final extraction. A fine powder was collected and used in the ORAC method (described later) to determine the antioxidant capacity. The antioxidant activity in grape seed and grape pomace extract were determined by using the ORAC method as described by (Cao and Prior 1999). 0.1 g GPE was dissolved in 50 ml (95 percent ethanol and distilled water), then 5 ml from the solution was diluted with 50 ml (95 percent ethanol and distilled water). The dilution was

diluted again with a suitable buffer solution. The dilution was analyzed using the ORAC (HTS 7000 plus bio assay reader, Perkin Elmer) to determine antioxidant capacity.

Fig. 1. Schematic representation of the extraction procedure for separating antioxidant from grape pomace.



The Experiment

Natural antioxidants and chemicals

Grape seed extract, known as GSE, containing 95 percent proanthocyanidins standardized extract powder, was obtained from Nusci (Walnut, CA 91788, USA). GSE was purchased for this study partly as a natural antioxidant for comparison purposes, and partly because of insufficient quantities of GPE form Oklahoma grapes for all experiments. The antioxidant capacity of GSE was evaluated using the ORAC analysis as described later. Grape pomace extracts (from Cynthiana grapes grown in Oklahoma) were prepared in the lab as previously mentioned. Synthetic antioxidant, butylated hydroxytoluene (BHT); 2, [6]-Di-tert-Butyl-p-cresol) was obtained from SIGMA (B1378, Louis, MO, USA).

Experimental design

Figure (2) shows the process was used to make meat goat meat sausages products used in this study. GSE, GPE, and BHT, were added individually to minced goat meat in order to evaluate and compare their effects in meat system. Treatments used in this research were formulated as follows: (1) control (no antioxidant added); (2) 1.135 g BHT/ 5 kg of sausage (3) 5.625 g GSE/ 5 kg of sausage and, (4) 3.405g GPE/ 5 kg of sausage. Products were made, dried, and then stored at 4° C for 28 days. Analyses included thiobarbituric acid-reactive substances (TBARS), instrumental color, proximate

analysis, microbial analysis, and sensory evaluation for flavor and color. Data were statistically analyzed to compare the results.

Product manufacture

Boneless goat meat, from the shoulder, and goat fat were obtained fresh from the Oklahoma City meat market. Sausage was formulated to contain 70 percent goat meat, 30 percent goat fat, and 2.7 % smoked polish kielbasa seasoning ingredients (Old plantation seasonings A.C. Legg, INC.). The seasoning ingredients consisted of a blend of salt, dextrose, spices, monosodium, glutamate (4.55 percent), garlic powder, and sodium erythorbate (6.20 g) mixed with 3 percent water.

Selected antioxidants were added for comparison to the control. The goat meat and goat fat were cut into approximate 5 cm cubes and stored at 4° C for 24 hours to drain off purge. Lean and fat meats were ground through a 13 mm plate Biro Grinder (Doerr, Cedarburg, Wisconsin, USA). Ingredients were added with 3 percent water and a Northern hand-cranked mixer (item 168676K, Northern Tool + Equipment, Burnsville, MN) was used in order to mix the ingredients for 3 minutes. The mixed ingredients were divided into four equal portions by weight. Natural and synthetic antioxidants were added individually. Each treatment was mixed by using a Northern hand-cranked mixer for 1.5 minutes.

Minced meat was reground through a 3.2 mm plate installed in a Biro grinder. A stuffer (VF 608 Handmann, 88400 Biberch Riss, Germany) was used to form the product

into1.25 × cm 15 cm long sausages, which were later sliced into patties (3.2 cm thick). The sausages were linked at about 15 cm. Figure 3 shows the four treatments after stuffing. The links were hung on a rack to prepare them for drying.

An ALKAR oven (53555, Alkar-rapidpak IncLodi, Wisconsin, USA) was set at a dry bulb temperature of 12°C, wet bulb temperature of 9.7°C and relative humidity (RH) of 75% to dry treatments for 24 hours, or until they lost about 7 - 8 percent of their initial weight as described by Soultos and others (2008). However, the goat sausage samples lost 12 percent of their initial weight after 20 hours due to a problem with the ALKAR oven. The main oven relay failed because there was no power to the refrigerator compressor. The product was placed in the oven for about 3-4 hours before the power failure was noticed. Product was placed into the cooler during repairs.

The sausages were then placed in hard polyester trays, wrapped with air-permeable polyethylene film (film wrapper machine, WH SS-L, Dallas Texas) and stored at 4° C for up to 28 days (Soultos and others 2008). TBARS, proximate analysis and microbial analysis were performed on days 0 and 28 except the sensory evaluation was measured only on day 0 due to sample spoilage caused by microbial growth during the storage period.

Fig. 2. Process used to make goat meat sausage in this study.

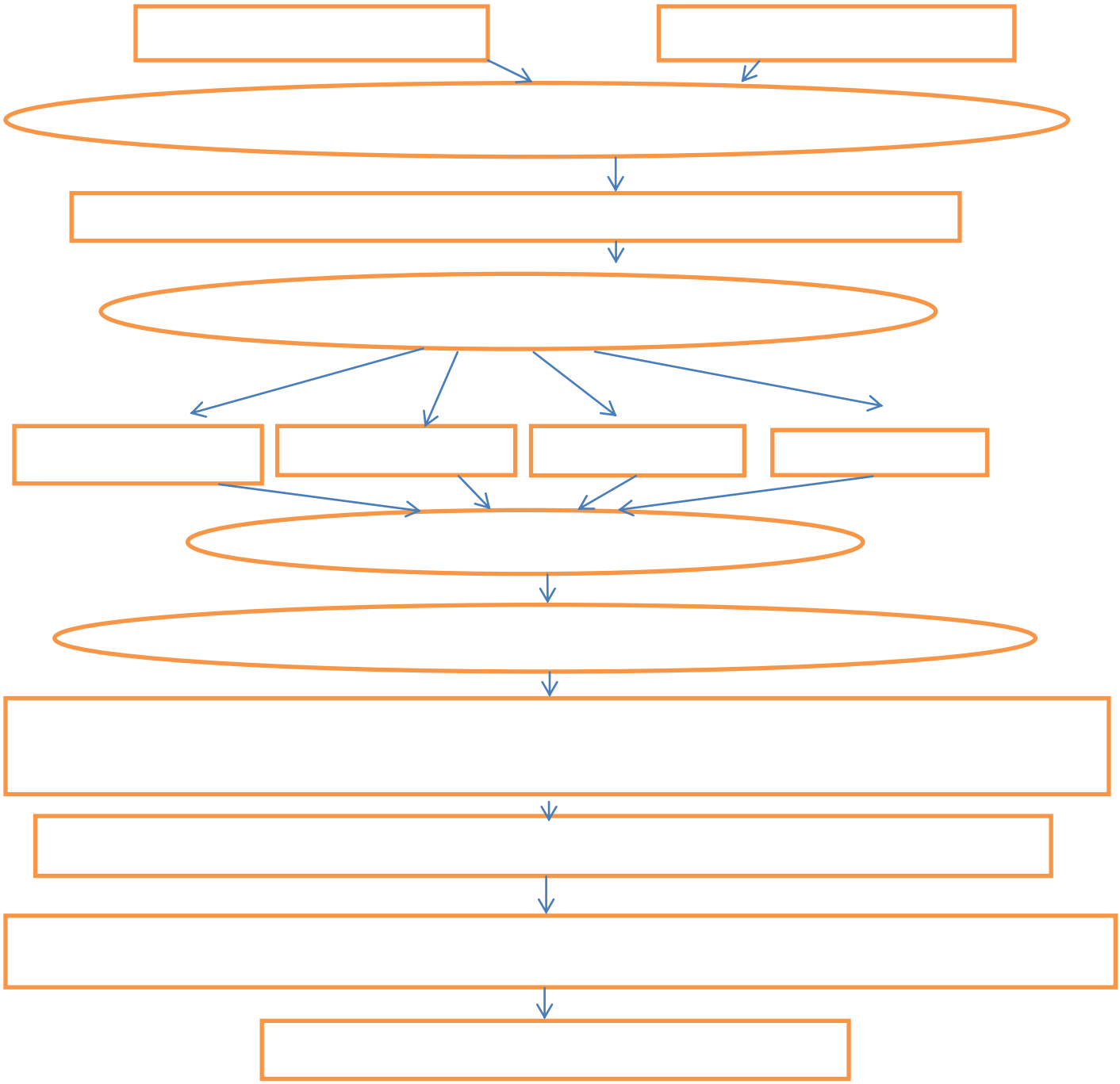


Fig. 3. Four goat sausage treatments were hung on racks for processing.

Laboratory evaluation methods:

Sensory evaluation

A thirty member consumer panel was recruited from faculty, staff and graduate students of the Food Science program and Animal Science department at Oklahoma State University. The panelists were asked to evaluate sample color, flavor, and off-flavor of cooked goat-meat sausage on a 10-point descriptive hedonic scale ranging from (1) to (10) (Sebranek and others 2005) on day 0 (see Appendix 1).

Before the test, panelists were familiarized with sensory terms and product ingredients. Panelists were stationed in isolated booths, and the sausage samples were evaluated under fluorescent lighting. The panelists were instructed to consume water and unsalted crackers between samples to cleanse their palates. Sausages were grilled for 8 minutes at 176.7°C on an electrical plate grill (XLT, Dart controls, micro-drive oven, Wichita, KS, USA) to a core temperature of 72 to 76°C.

A thermocouple was used to check the temperature in the center of the samples by inserting through the side. Samples were held in a food warmer (Crystal lake, PS-122015, IL USA) for 30 min before evaluation in order to keep the sausages warm. Sausage slices were cut into quarters, approximately 20 g each, placed on paper plates and served to panelists. Sausage samples were identified with random 3-digit codes. Products were

scored for color, ranging from pink to brown. In addition to color panelists were asked to score flavor for ‘‘goat’’ flavor (no goat flavor/ intense goat flavor) and ‘‘warmed over’’ flavor (no warmed-over flavor/intense warmed-over flavor). All data were collected and statically analyzed. The effect of each treatment on color and flavor was assessed with analysis of variance assuming a randomized complete block design.

Proximate analysis

Proximate analysis was completed on goat sausage samples on day 0 (only the control was measured) and day 28 (all sample treatments were measured). Only the sample control was measured on day zero because the levels of moisture, protein, ash, and fat are almost the same for all treatments with the only difference being the type of antioxidants added. Moisture, ash, protein and fat content were determined. The goat meat sausages were frozen and packed under vacuum for analysis at a later date (day zero and day 28). Frozen sausages were grounded using a coffee grounder (80365 Hamiltton Beach, Proctor Silex, customer service, USA). Moisture content was determined in duplicate by drying a 2 g minced sample at 102 C for 16-18 hours in a forced-air oven (Equatherm oven, Curtin Matheson Scientific, Inc.) (Robert and Bradley 2010).

Ash content was determined in duplicate by burning a 3 g sample of ground product in a muffle furnace (Furnatrol, Sybron/ Thermolyne) at 550 C for 6 hours (Marshal 2010). Protein was evaluated using the Kjeldal method (2400 Kjeltec Analyzer unit Foss TTECATOR). The protein conversion factor was assumed to be 6.25 (Chang

2010). Fat was analyzed according to AOAC 1992 method. the extraction with petroleum ether using the Sohxlet method was used. Figure 4 shows these instruments which used to determine proximate analysis.

Fig 4. Proximate analysis instruments: from left to right, muffle furnace, forced air oven, Sohxlet extractor and Kjeldal equipment.

Color determination

Color measurement was completed to determine if the color of the goat sausages changed over time. Surface color measurements were determined using a CM-3500d spectrophotometer or Chromameter (Minolta Co., Ltd. Osaka, Japan), which consisted of a measuring head, with a 30 mm diameter measuring area. The spectrophotometer was calibrated on the color space system using a black zero calibration box and a white calibration plate. Around 10 grams of peeled sausage samples were measured in a clear plate. The ‘L*’ value represents lightness and ‘a*’ and ‘b*’ values represent redness and yellowness, respectively. Color measurements were taken on the goat sausage samples on days 0, 15, and 28.

Fig 5. The spectrophotometer or Chromameter instrument used to measure the color of goat-meat sausage samples.

Microbial analysis

After peeling, sausage samples (10 g each) were cut aseptically into slices on a sterile cutting board. The samples were transferred aseptically to filter bags (Seward, BA 6041/STR filter bagsX10) and homogenized with 90 ml of 0.1% peptone water. The mixtures were homogenized for 1 min in a stomacher laboratory mixer (Lab blender, model 4000, Seward Circulattor) at 230 rpm. Mixtures were serially diluted (1:10) in 9 ml of 0.1% peptone water. From the resulting dilution, appropriate decimal dilutions were prepared, using the same diluent and plated on 3M™ Petrifilm™ aerobic count plate (3M Microbiology Products, St Paul, MN) in duplicate (Figure 6).

The Petrifilm™ Aerobic count plates were incubated in a horizontal position at 37 C^{o} for 30 hours (Ginn and others 1986). On day zero, dilution 3 was selected but dilution 5 was selected on day 28 because the count plate has high microorganism in day 28. The Bantex colony counter 920A was used to count the aerobic bacteria population. Average results of duplicate measurements are presented as log10 colony forming units (cfu/g) (Soultos and others 2008).

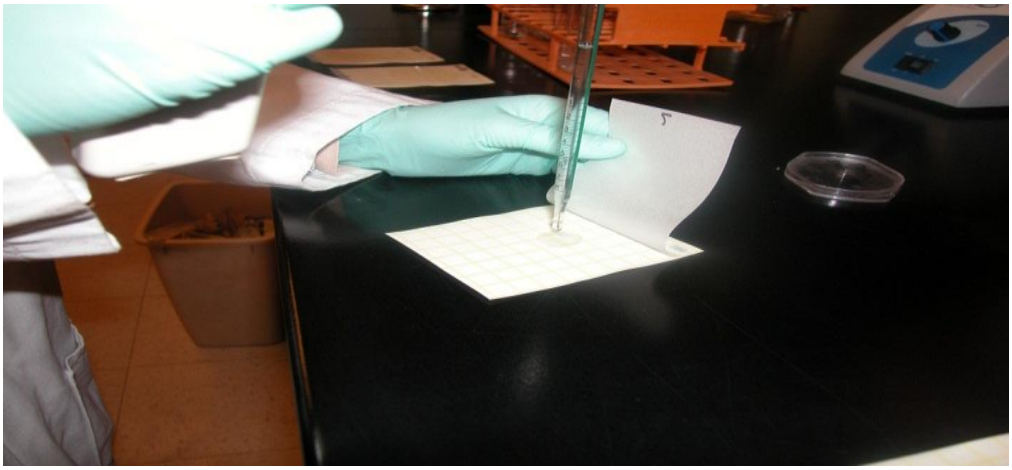


Fig 6. The dilution being plated on 3M™ Petrifilm™ aerobic count plate.

Measurement of lipid oxidation

Lipid oxidation was measured by the 2-thiobarbituric acid distillation procedure modified by Buege and Aust (1978) assay of lipid oxidation in muscle samples. Results were expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg. of malondialdehyde (MDA)/kg of muscle. TBARS values were measured in duplicate on days 0, 7, 14, 21 and 28 for fresh goat sausage samples.

CHAPTER IV

RESULTS AND DISCUSSIONS

Antioxidant activities of grape pomace and grape seed by ORAC

The ORAC activity of Oklahoma Cynthiana (Vitis Vinifera) grape pomace was calculated by adding the individual ORAC values measured for its seed and pomace powder. The antioxidant was extracted from the seed and pomace powders using a combination of ethanol and water. A two-step extraction with 200 ml of 50 percent ethanol solution and 150 ml 95 percent ethanol solution was found to be sufficient to extract the antioxidant contained in the grape pomace.

The ORAC activities measured triplicate (n=3) for the commercial grape seed extract (95 percent proanthocyanidins standardized extract powder) were significantly higher than grape pomace extract (P=0.0156). The ORAC activities of grape seed and grape pomace extracts are shown in Table 2. The grape seed extract has around 802.6 µmol trolox eq. /ml/g dry matte which was higher than grape pomace extract 712.1 µmol TE/g dry matters. Good cultivar quality of commercial grape seed or purity of phenolics, which have a higher antioxidant activity, might be a reason why the ORAC value for the commercial GSE was higher than GPE.

Results agree with previous research by Yilmaz and Toledo (2006) who measured ORAC with phycoerythrin ($ORAC_{PE}$) values for grape seed and skin and showed that $ORAC_{PE}$ values in grape seed are 3 to10 times more than grape skin samples on a dry basis ($p<0.05$). Chardonnay seed had an $ORAC_{PE}$ value of 637.8 µmol TE/g

dry matter which has higher $ORAC_{PE}$ values than both merlot skin (70 µmol TE/g dry matter) and chardonnay skin (103 µmol TE/g dry matter), because grape seed extracts have higher oligomeric or polymeric procyanidins than skins.

The current ORAC results for GSE and GPE were higher than several plant extracts. For example, Wang and others (1996) measured ORAC activity for several plants and found that ORAC value for strawberry was 154 µmol TE/g dry matter which is higher than plum, orange, whole red grape, kiwi fruit, whole pink grapefruit, whole white grape, banana, apple, pear, and melon (80, 52, 36, 36, 48, 26, 9, 13, 38, 10, and 13) µmol TE/g dry matter, respectively.

Also, Wang and Lin (2000) found ORAC values in fruit and leaves of strawberry, raspberry, and blackberry varies with cultivar and development stage. The ORAC values for black raspberry (Jewel cultivar) green stage is around 162 µmol TE/g dry matter which is higher than strawberry and blackberry in different stages. T test was performed to compare between GPE and GSE for ORAC values.

Table 2. Measured ORAC values for Cynthiana (Vitis Vinifera) grape pomace and commercial grape seed powders.

Type	Mean (n=3) ORAC µmol TE/g dry matter	Stander error ORAC	PVALUE ORAC
GPE	712.1 [b]	8.1	0.0156
GSE	800.6 [a]	20.3	

[abc] mean values with different superscripts letters within a column are significantly different at $p<(0.05)$.

Sensory analysis

Sensory panel results are shown in Table 3 a., b., and c for the effect of different treatments on goat sausages on day 0. No significant differences occurred in the intensities of the off –flavor and goat flavor scores across the different treatment applied to the cooked goat meat sausages; (P=0.4193), (P=0.2527) respectively. The sensory scores for off- flavor were ranged from 1.6 to 2 and goat flavor scores ranged from 5.1 to 5.8 on day zero.

It was concluded that the different treatments of GSE, GPE, BHT, and control didn't affect the goat flavor and off flavor of cooked goat sausages on day 0 at the levels employed in the present study. However, different treatments had a significant effect on the color response (p=0.0047). The goat-meat sausages containing GSE had a higher brown color score (8.0) than GPE, BHT, and control. The reason might be that the GSE at this level (5.625 g/5 kg goat meat) was high enough to clearly appear in the product followed by GPE containing sample.

Also, the color pigment in GSE and GPE might be the reason the brown color appeared in the product (see table 3-a). Microbial growth was the reasons why the 28th day sensory analysis didn't occur in the current study. Effect of treatment on color and the flavors was assessed with analysis of variance assuming a randomized complete block design. Panelist were considered the blocking variable. Presented below are the means and standard errors for each treatment. Treatment effect was significant for the color response ($p=0.0047$). A multiple comparison for pairwise comparisons of the treatments was conducted. Two means with the same letter are not significantly different at a 0.05 level of significance. Note that the flavor means were not significantly different, thus no treatment effect for these responses.

Table 3.a. Sensory attributes (color) for fresh goat-meat sausages with different antioxidants.

Treatment	Mean brown color (n=30)	Stander error color	P -value
BHT	6.8 b	0.3	0.0047
Control	6.9 b	0.3	
GPE	7.3 ab	0.3	
GSE	8.0 a	0.3	

Table 3.b. Sensory attributes (goat flavor) for fresh goat-meat sausages with different antioxidants.

Treatment	Mean Goat flavor (n=30)	Stander error Goat flavor	Overall p- value
BHT	1.9 a	0.5	0.48269
Control	5.2 a	0.5	
GPE	5.9 a	0.5	
GSE	5.1 a	0.5	

Table 3.c. Sensory attributes (off flavor) for fresh goat-meat sausages with different antioxidants.

Treatment	Mean off flavor (n=30)	Stander error off flavor	Overall p-value
BHT	1.9 a	0.2	0.4193
Control	2.0 a	0.2	
GPE	1.7 a	0.1	
GSE	1.9 a	0.2	

[abc] mean values with different superscripts letters within a column are significantly different at $p<(0.05)$.

Proximate analysis

The results for the proximate analysis are shown in Table (4-a) and (4-b). Proximate analysis was measured on day 0 for the control only, because the levels of moisture, protein, ash, and fat are almost the same for all treatments. The only difference between the four treatments was the type of antioxidants added. Proximate analysis indicated that fresh sausages (day 0) had the highest moisture content (44.4 percent) which might be related to the higher water content and the water holding capacity of fresh sausages (Table 4-a).

The moisture content for all goat sausage treatments ranged from 40.30 to 41.79 in day 28 (Table 4-b). Although the moisture content for all sausage samples was less on day 28 when compared with day 0, there was no significant difference between all treatments and the moisture content for all sausage samples. The reason might be related to a loss of moisture during refrigerated storage.

On the 28th day, protein (16.2–17.8%), fat (32.2–36.2%) and ash (2.7–3.0%) contents were significantly increased ($p < 0.05$), while the moisture contents (40.3–41.7%) decreased compared to day 0. As expected, ash, Protein and fat contents were slightly increased in all treatments when compared to day 0. The differences which were detected in protein and fat contents between treatments at day 0 and 28 might be partially explained by the differences in moisture content. The highest moisture content corresponds to the lowest protein content, and the lowest fat content (Table 4-a). However, protein and fat contents are significantly different between treatments on day

28 (Table 4-b). The higher ash contents were found in samples treated with GSE, BHT, and GPE respectively. Analysis was done in two parts. First, comparisons of control values for the two days were done with independent t- tests. Means and standard errors are presented.

Table 4. a. proximate analysis of control treatment of the sausage samples on day 0 and 28.

Day	Mean Moisture % (n=2)	Stander error moisture	p- value
0	44.4 a	0.2	0.0017
28	40.8 b	0.3	

Day	Mean protein % (n=2)	Stander error protein	p-value
0	15.4 a	0.2	0.1722
28	16.2 a	0.5	

Day	Mean Ash % (n=2)	Stander error ash	p-value
0	2.7 b	0.01	0.0280
28	2.8 a	0.01	

Day	fat % (n=1)	Stander error fat	p-value
0	32.2	. 0.0003	
28	34.3		

[abc] mean values with different superscripts letters within a column are significantly different at $p<(0.05)$.

Next, comparisons of day 28 values for the different treatments were conducted with analysis of variance. The p-value reflects the overall p-value given by ANOVA.

Table 4. b. Results of proximate analysis tests on different sausage treatment samples for day 28.

Treatment	Mean moisture % (n=2)	Stander error moisture	P-VALUE
BHT	41.8 a	0.2	0.1618
Con	40.8 a	0.3	
GPE	41.4 a	0.6	
GSE	40.4 a	0.09	

Treatment	Mean protein % (n=2)	Stander error protein	P-VALUE
BHT	17.1 ab	0.0006	0.0479
Con	16.2 b	0.5	
GPE	17.6 a	0.01	
GSE	17.8 a	0.2	

Treatment	Mean Ash % (n=2)	Stander error ash	P-VALUE
BHT	2.8 b	0.02	0.00110
Con	2.8 c	0.01	
GPE	2.8 bc	0.01	
GSE	3.1 a	0.001	

Treatment	Fat % (n=1)	Stander error Fat	P-VALUE
BHT	36.3 a	.	0.0003
Con	34.3 c	.	
GPE	35.2 b	.	
GSE	34.0 d	.	

[abc] mean values with different superscripts letters within a column are significantly different at $p<(0.05)$.

Surface color

Color measurements were taken on the goat-meat sausage samples on day 0, 15, and 28. Color was evaluated to detect if different antioxidant treatments caused any changes in raw goat-meat sausages during the shelf life. Results for the color score are listed as L*, a*, and b* numbers. The L* value was a measure of darkness on a scale from 0 (lightest) to 100 (darkest). The a* value measures red to green color, and the b* value measures yellow to blue color. The data was collected, transferred to an Excel file, and analyzed. Tables 5-7 show the results after the analysis. Table 5 shows analysis of

the L* value, Table 6 shows the a* value, and Table 7 shows the b* value. In these Tables, means with the same letter are not significantly different but means with different letters in the same row are significantly different ($P \leq 0.05$).

The color (a*) for redness are shown in Tables 7 a, and b. On day 15, the redness color increased for samples treated with GSE and GPE, in contrast to BHT compared to control. This is possibly due to the effect of natural pigment in the GSE and GPE itself. There was significant color loss (decrease in redness) over time (day 28). So, all the treatments had significant effect on decrease redness of the goat sausages except the control samples were increased over time. This reduction in (a*) values might be due to oxygenation of meat myoglobin.

Similar results were reported by Garrido and others (2011) Pigment oxidation can still occur even if different types of antioxidants are added to pork patties. This finding agreed with Rababah et al. (2011). Rababah found that the redness of the goat meat decreased for the control and treated (included grape seed and greet tea extracts) meat samples during 9 days shelf life period. Also, the color changes might be related to microbial growth because microbial growth can affect color through oxygen consumption as reported by Kusuma (2008). No significant differences in (a*) redness values, as a result of GSE and GPE at the level applied in current study, were observed over the 28 day refrigerated storage period.

In addition, the effect of treatments and time effect on b* values were significant (Table 6 a., and b). In general the b* values of all samples decreased ($p<0.05$) slightly from day 0 to day 28. However, GSE and GPE containing samples had no significant difference on day 28 of the shelf life study.

In general, L* values of all samples increased over time. For example, the control increased from about 50 to about 52 after 15 days of storage. GPE-containing samples also increased about 3 L* units during the 15 and 28 days storage period indicating that they were became lighter as well. However, after 28 days of storage L* values of samples containing GSE had no significant different. At the initiation of the study, the GSE-containing sample had higher L* values than the other treatments. Moreover, L* values decreased in the sample with GSE until day 28. This trend did not happen for the GPE, BHT, and control samples. The L* values on day 0 for GPE, BHT and control were lower than those on day 15 and 28 (see Tables 5- a and b). Comparisons made by ANOVA procedures. Many of the comparisons involving day 0 data are suspect due to the lack of any replication. Below are comparisons of treatments given day.

Table 5. a. L* values measured for the goat-meat sausage samples compared between treatments over time.

Day	Treatment	Mean L* (n=3)	Stander error L*	P -value
0	BHT	50.1 b	.	0.0003
0	Con	50.5 b	.	
0	GPE	49.5 b	.	
0	GSE	52.2 a	.	

Day	Treatment	Mean L* (n=3)	Stander error L*	P -value
7	BHT	52.4 a	0.02	0.0618
7	Con	52.1 a	0.003	
7	GPE	52.0 a	0.32	
7	GSE	51.6 a	0.07	

Day	Treatment	Mean L* (n=3)	Stander error L*	P -value
28	BHT	52.9 a	0.14	0.0008
28	Con	52.1 b	0.03	
28	GPE	53.0 a	0.2	
28	GSE	51.8 b	0.9	

[abc] mean values with different superscripts letters within a column are significantly different at $p<(0.05)$.

Table 5. b. L* values measured for the goat-meat sausage samples compared same treatments on different times.

Day	Treatment	Mean L* (n=3)	Stander error L*	P -value
0	BHT	50.1 b	.	<.0001
15	BHT	52.4 a	0.02	
28	BHT	52.9 a	52.9	

Day	Treatment	Mean L* (n=3)	Stander error L*	P -value
0	Con	50.5 b	.	0.0020
15	Con	52.1 a	0.003	
28	Con	52.1 a	0.03	

Day	Treatment	Mean L* (n=3)	Stander error L*	P -value
0	GPE	49.5 c		<.0001
15	GPE	52.0 b	0.3	
28	GPE	53.0 a	0.2	

Day	Treatment	Mean L* (n=3)	Stander error L*	P -value
0	GSE	52.2 a		0.3295
15	GSE	51.6 a	0.07	
28	GSE	51.8 a	0.9	

[abc] mean values with different superscripts letters within a column are significantly different at $p<(0.05)$.

Table 6. a. b* values measured for the goat-meat sausage samples compared between treatments over time.

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Day	Treatment	Mean b * (n=3)	Stander error b*	P- Value
0	BHT	15.1 b	.	0.0199
0	Con	16.7 a	.	
0	GPE	16.1 ab	.	
0	GSE	15.5 b	.	

Day	Treatment	Mean b * (n=3)	Stander error b*	P- Value
15	BHT	15.4 a	0.2	0.0021
15	Con	14.5 b	0.1	
15	GPE	15.6 a	0.2	
15	GSE	14.9 b	0.07	

Day	Treatment	Mean b * (n=3)	Stander error b*	P- Value
28	BHT	14.6 c	0.3	0.0025
28	Con	15.8 a	0.1	
28	GPE	15.3 b	0.3	
28	GSE	15.2 b	0.3	

[abc] mean values with different superscripts letters within a column are significantly different at $p<(0.05)$.

Table 6. b. b* values measured for the goat-meat sausage samples compared same treatments on different times.

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Day	Treatment	Mean b* (n=3)	Stander error b*	P- Value
0	BHT	15.1 ab	.	0.0193
15	BHT	15.4 a	0.2	
28	BHT	14.6 b	0.3	

Day	Treatment	Mean b* (n=3)	Stander error b*	P- Value
0	Con	16.7 a	.	<.0001
15	Con	14.5 c	0.1	
28	Con	15.8 b	0.1	

Day	Treatment	Mean b* (n=3)	Stander error b*	P- Value
0	GPE	16.1 a		0.1131
15	GPE	15.6 a	0.2	
28	GPE	15.3 a	0.2	

Day	Treatment	Mean b* (n=3)	Stander error b*	P- Value
0	GSE	15.5 a	.	0.2183
15	GSE	14.9 a	0.07	
28	GSE	15.2 a	0.1	

[abc] mean values with different superscripts letters within a column are significantly different at $p<(0.05)$.

Table 7. a. a* values for the goat-meat sausage samples:.

Day	Treatment	Mean a* (n=3)	Stander error a*	P- value
0	BHT	10.9 a	.	0.9184
0	Con	10.9 a	.	
0	GPE	11.0 a	.	
0	GSE	10.6 a	.	

Day	Treatment	Mean a* (n=3)	Stander error a*	P- value
15	BHT	9.7 a	0.20	<.0001
15	Con	9.9 a	0.007	
15	GPE	7.5 b	0.3	
15	GSE	7.2 b	0.2	

Day	Treatment	Mean a* (n=3)	Stander error a*	P- value
28	BHT	8.6 c	0.3	<.0001
28	Con	12.1 a	0.1	
28	GPE	9.3 b	0.3	
28	GSE	9.7 b	0.3	

[abc] mean values with different superscripts letters within a Colum are significantly different at $p<(0.05)$.

Table 7. b. a* values for the goat-meat sausage samples.

Day	Treatment	Mean a*	Stander error a*	P- value
0	BHT	10.9 a	.	0.0004
15	BHT	9.7 b	0.2	
28	BHT	8.6 c	0.3	

Day	Treatment	Mean a*	Stander error a*	P- value
0	Con	10.9 b	.	<.0001
15	Con	9.9 b	0.007	
28	Con	12.1 a	0.1	

Day	Treatment	Mean a*	Stander error a*	P- value
0	GPE	11.0 a	.	<.0001
15	GPE	7.5 c	0.3	
28	GPE	9.3 b	0.3	

Day	Treatment	Mean a*	Stander error a*	P- value
0	GSE	10.6 a	.	<.0001
15	GSE	7.2 b	0.2	
28	GSE	9.7 a	0.3	

[ab] mean values with different superscripts letters within a column are significantly different at $p<(0.05)$.

Microbial growth

In general, fresh sausages have a pH of around 5.5 and a water activity higher than 0.97. Therefore, fresh sausages are perishable products (Kusuma 2008). The spoilage point is 7-8 $\log_{10}$ CFU/g in fresh sausages (Kusuma 2008). On day 0, the total microbial count for all treatments reached a maximum of 5.2 $\log_{10}$ CFU/g in fresh goat sausages. In the day of the process, The total microbial count result was slightly high compared to other study such as Garrido and others (2011) who found that Total Viable Count (TVC) for the control samples in pork burgers was 4.3 $\log_{10}$ CFU/g in day 0. the reason why was slightly high in day zero in this study might be the process took more than 20h due to Alkar oven problem as explained previously. On day 28, microbiological counts on over- wrapped sausages were significantly higher than on day 0 samples as shown in Fig. 7. Day 28 samples reached a value greater than 7 CFU/g during the shelf life storage except GPE treatments at 4 C°. Samples with GPE had the lower total aerobic bacteria population which was 6.9 $\log_{10}$ CFU/g. Although natural antimicrobial occurs in grape pomace (Özkan and others 2004), the amount applied in this research might be not enough to inhibit the microbial growth.

Microbial population (log c.f.u./g) of total aerobic bacteria in the four types of sausages packed under aerobic conditions for 28 days are listed in Tables 8 a and b. Generally, the treatments with GSE, BHT, and GPE did not affect the spoilage of goat sausages. This result agrees with previous studies which found that the addition of red grape pomace extract didn’t inhibit the spoilage of burgers during 6 days of storage under aerobic conditions (Garrido and others 2011). Carpenter and others (2007) found adding

of GSE and BB did not significantly improve the microbial status of pork patties Furthermore the growth values for 0 day of storage ranged from 5. 21 to 5.23 log c.f.u./g. Therefore, in goat-meat sausages with GSE, GPE, and BHT at the level which was tested in the study did not prevent spoilage during storage completely. So, the packaging systems might be important in sausage preservation. Also, the antimicrobial effect of these natural extracts may increase when they are added in higher concentrations to food systems (Garrido and others 2011).

Table 8. a: Statistical analysis of population data of microbes on fresh goat-meat sausage on day 0 and 28 of the shelf-life study at 4° C between different treatments.

Day	treatment	Mean log microbial	Stander error log microbial	P-value
0	BHT	5.24 a	0.06	0.9916
0	CON	5.23 a	0.01	
0	GPE	5.24 a	0.01	
0	GSE	5.22 a	0.03	

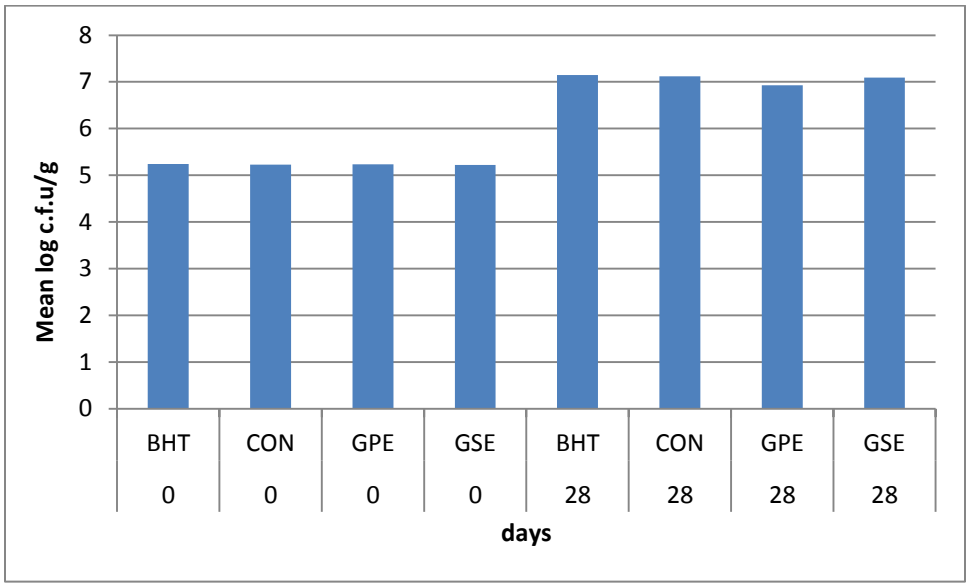
Day	treatment	Mean log microbial	Stander error log microbial	P-value
28	BHT	7.15 a	0.03	0.0406
28	CON	7.12 a	0.04	
28	GPE	6.92 b	0.02	
28	GSE	7.09 a	0.1	

Table 8. b. Statistical analysis of population data of microbes on fresh goat-meat sausage on day 0 and 28 of the shelf-life study at 4° C.

Day	treatments	Mean log microbial	Stander error log microbial	P-value
0	BHT	5.24 b	0.062469	<.0001
28	BHT	7.14 a	0.03	
0	CON	5.23 b	0.01	
28	CON	7.11 a	0.04	
0	GPE	5.24 b	0.01	
28	GPE	6.92 a	0.02	
0	GSE	5.22 b	0.03	
28	GSE	7.09 a	0.1	

[ab] within each day (each antioxidant type compared to the control) mean values in the same column and table bearing different superscripts are significant different, *p<(0.05).*

Fig. 7. Effect of different preservative treatments on microbial growth in goat-meat sausage samples on days0 and 28 of a shelf life study.



TBAR analysis

Tables 9-a and 9-b illustrate the effects of the different treatments and storage time on lipid oxidation levels (thiobarbituric acid reactive substances TBARS measured as mg malondialdehyde/ kg in raw- refrigerated goat-meat sausage samples over a 28 day period. The results showed that TBARS values for 0-28 days of refrigerated storage ranged from 0.41 to 0.38 mg malondialdehyde/ kg goat meat of total product dry basis. Meant BARS values for the control samples increased with increasing refrigerated storage time from 0.40 at day 0 to 0.41 mg malondialdehyde / kg on day 14. However, after day 14, the control started to decrease until values reached 0.39 mg malondialdehyde / kg on day 28 as shown in Table 9-b. Natural antioxidants in the

sausage ingredients may have helped to decrease the TBAR value in the control sausages after 3 weeks so this was not unexpected. On day 0 and 14, mean TBARS values for all treatments had result that were not significant different except BHT was significantly different, 0.40 and 0.39 mg malondialdehyde / kg respectively.

After 28 days of refrigerated storage, GSE (5.625 g) was the most effective antioxidant *($p<0.05$).* Mean TBARS formation was significantly lower for products prepared with GSE during the entire experiment when compared to the control or treatment with GPE, and BHT. The reason might be that the GSE had higher antioxidant activity at the higher level of plant extract.

This result is in agreement with previous results. For example, Carpenter and others (2007) reported that GSE (400µg/g, 1000 µg/g) significantly improved the oxidative stability of cooked pork patties after 12 d shelf life. Similarly, Kulkarni and others (2011) reported that in pre-cooked, frozen, re-heated beef sausages, the samples containing 100 and 300 ppm GSE were protected against oxidation during a 4 month storage period. Sausage containing (3.40 g/5kg muscle sausage) GPE also maintained significantly lower mean TBARS values compared to the control after 21 days. Similarly, samples containing BHT (1.13g//5kg muscle sausage) had lower mean TBARs values in day 21 compared to all treatments and control. When data were pooled over storage time, TBARS were affected by antioxidant (Table 9-a and 9-b). In general, mean TBARS value for all treatments increased until reached day 14 but, on day 21 and 28 mean TBARS value decrease compared to control in goat meat sausages.

Table 9. a. Effect of grape seed extract (GSE), grape pomace extract (GPE) and (BHT) compared to control on lipid oxidation (TBARS) in in raw- refrigerated goat-meat sausages.

Day	Treatments	TBA (mg/kg)	Stander error	P- value
0	BHT	0.40 b	.0008	.0003
0	CON	0.41 a	.0008	
0	GPE	0.41 a	.0001	
0	GSE	0.41 a	.001	

day	Treatments	TBA (mg/kg)	Stander error	P- value
7	BHT	0.40 b	.001	.0214
7	CON	0.41 b	.0002	
7	GPE	0.41 a	.0008	
7	GSE	0.41 ab	.001	

day	Treatments	TBA (mg/kg)	Stander error	P- value
14	BHT	0.39 b	.002	<.0001
14	CON	0.41 a	.002	
14	GPE	0.41 a	.001	
14	GSE	0.41 a	.0006	

day	Treatments	TBA (mg/kg)	Stander error	P- value
21	BHT	0.39 c	.001	.0002
21	CON	0.40 a	.0008	
21	GPE	0.4 b	.001	
21	GSE	0.39 bc	.0006	

28	BHT	0.39 a	.004	.0107
28	CON	0.39 a	.0008	
28	GPE	0.40 a	.003	
28	GSE	0.38 b	.002	

[abc] within each day (each antioxidant type compared to the control) mean values in the same column and table bearing different superscripts are significant different, *p<(0.05).*

Table 9. b. Effect of each treatment on TBARS value over time.

day	treatments	TBA (mg/kg)	Stander error	P- value
0	BHT	0.40 b	.0008	<.0001
7	BHT	0.40 a	.001	
14	BHT	0.39 cd	.002	
21	BHT	0.38 d	.001	
28	BHT	0.39 bc	.004	

day	treatments	TBA (mg/kg)	Stander error	P- value
0	CON	0.40 bc	.0008	<.0001
7	CON	0.41 b	.0002	
14	CON	0.41 a	.002	
21	CON	0.40 c	.0008	
28	CON	0.39 d	.0008	

day	treatments	TBA (mg/kg)	Stander error	P- value
0	GPE	0.41 a	.0001	<.0001
7	GPE	0.41 a	.0008	
14	GPE	0.41 a	.001	
21	GPE	0.39 b	.001	
28	GPE	0.40 b	.003	

day	treatments	TBA (mg/kg)	Stander error	P- value
0	GSE	0.41 b	.001	<.0001
7	GSE	0.41 ab	.001	
14	GSE	0.41 a	.0006	
21	GSE	0.39 c	.0006	
28	GSE	0.38 c	.002	

[abc] within each day (each antioxidant type compared to the control) mean values in the same column and table bearing different superscripts are significant different, *p<(0.05).*

CHAPTER V

CONCLUSION

Briefly, the results of this research show the following:

1. Commercial grape seed extract indicated higher antioxidant capacity than Oklahoma Cynthiana (Vitis Vinifera) grape pomace extract as described in Table 2.
2. Although sausages containing GSE had higher levels of brown color followed by samples containing GPE, flavors and off- flavors were not significantly different between treatments for fresh goat sausages on the first day of processing (see Table 3). Sensory testing was not conducted on day 28 because of excessive microbial growth/contamination in samples.
3. On day 28, protein, fat, and ash contents of assorted meat sausage samples were significantly increased, while the moisture content decreased compared to day 0 (see Table 4).
4. In general, there was significant color loss (decrease in redness and yellowness) over time in all meat samples. However the L* value increased over time (see Tables 5, 6, and 7).

5. GSE and GPE added to goat meat sausages at levels of 5.625 g/5 kg, and 3.405 /5 kg goat sausage (respectively) did not affect the microbial spoilage compared to control samples (see Table 8).
6. The effect of GSE (5.625 g/ 5 kg goat sausages) and GPE (3.405 g/ 5 kg goat sausages) on oxidative stability of goat meat sausages stored at 4 C^{o} for 28 days was significantly greater compared to the control samples. When TBAR values of GSE, GPE and BHT were compared to the control samples, GSE showed the best result in the antioxidant activity for raw goat meat sausages, followed by GPE (see Table 9).
7. The shelf life of the goat sausage was increased by using GSE and GPE compared to the control. However the antimicrobial activities of the GSE and GPE were not enough to prevent microbial growth that resulted in spoilage of samples during this study.

RECOMMENDATIONS

This research faced some limitations that caused some inaccurate results. For example, TBARS values were lower than 0.41380 mg malondialdehyde/ kg goat for all treatments including control samples. For the results to be more accurate, it is recommended that future researchers follow new steps to enable better results for further analysis. Steps that should be followed are listed below:

1. The GSE and GPE were more efficient at decreasing lipid oxidation when compared to the controls. However, the TBARS values were lower than 0.41380 mg malondialdehyde/ kg goat meat for all treatments including the controls in this study. The ingredients that were added and provided as readymade affected the TBAR result by affecting lipid oxidation. Therefore, the effect of the natural antioxidant which was added to the goat meat sausages was not demonstrated. The 2.7 % smoked polish kielbasa seasoning ingredients include a blend of salt, dextrose, spices, monosodium glutamate (4.55 %), garlic powder, and sodium erythorbate (6.20 g). All these ingredients acted as an antioxidant source which affected the resulting quality. For example, sodium erythorbate (6.20 g) which is structurally related to vitamin C is considered as an antioxidant. Also, garlic powder is an antioxidant source.
2. It is important to measure the anti-microbial effect of the natural antioxidants (GPE) and (GSE) before adding them to goat meat sausages. Using an inhibition zone method to determine the amount of antimicrobial effect in natural antioxidants might help researchers to know the concentration of GSE and GPE to be added to the goat meat sausages in order to prevent microbial growth.

3. Higher concentrations of GSE and GPE may lead to better results for increasing the shelf life and preventing microbial growth. Comparisons between different concentrations may show the range for higher and lower antimicrobial activities for natural antioxidants. Moreover, the different natural antioxidant concentration ranges on goat meat sausages may show the lipid oxidation range and give the optimal concentration among different antioxidant treatments and concentrations for products.

4. The kind of packaging might be part of the reason for spoilage during the refrigerated storage period. Therefore, different packaging methods may show an increase in the shelf life for meat products. Vacuum packaging is a good example of controlling the environment to prevent microbial and oxidation problems.

5. Microbial analysis on day zero revealed high numbers.. The meat may have been contaminated by careless handling before being obtained for use in this study.

6. Finally, there were limitations in funding, samples, and time available, which possibly led to less accurate results. Obtaining data from duplicate and triplicate samples would show better statistical results and illustrate significant differences between different treatments. For example, fat analysis was done only one time because the analysis was expensive. Better control methods would provide better samples statistically improved results

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APPENDICES

Appendix- 1 SAUSAGE EVALUATION FORM

Panelist number: ---------- Date: ----------

Test code: ----------

1- Color:

Pink

brown

1 2 3 4 5 6 7 8 9 10

2- flavor:

No goat flavor intense goat flavor

1 2 3 4 5 6 7 8 9 10

3- : Off-flavor

No warmed-over flavor

intense warmed-over flavor

1 2 3 4 5 6 7 8 9 10

PANELIST DATA FORM

Panelist number (assigned): ----------

1- Have you participated in sausage tasting competition? YES ---------- NO ----------

2- Do you take any medications affecting your taste and smell? If YES, explain:

3- Do you smoke on regular basis? YES ---------- NO ----------

4- Are you allergic to any food or food additives? YES ---------- NO ----------

5- Do you have any smell allergies? YES --------- NO ----------

6- Is your ability to distinguish tastes?

Better than average ---------- Average ---------- Below average ----------

7- Is your ability to distinguish odors?

Better than average ---------- Average ---------- Below average ----------

GENERAL INFORMATION

Steps to protect confidentiality of subjects:

1- Names of subjects are not recorded.
2- Subjects are assigned random, unique numbers from a list to track responses.
3- No photographs or digital images are taken of subjects.

Consent process:

1- Verbally inform subjects that their identity will not be recorded and is not needed for the purposes of the test.
2- Provide copies of the consent form to each subject.
3- Read the consent form to subjects and answer any questions.

Oklahoma State University Institutional Review Board

Date:	Wednesday, July 25, 2012
IRB Application No	AG1232
Proposal Title:	Evaluating The Effectiveness of Grape Seed Extract as an Antioxidant in Goat Meat Sausages
Reviewed and Processed as:	Exempt

Status Recommended by Reviewer(s): Approved Protocol Expires: 7/24/2013

Principal Investigator(s):

Ban Al Hakeem
126 FAPC
Stillwater, OK 74078

Tim Bowser
124 FAPC
Stillwater, OK 74078

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

☒ The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be submitted with the appropriate signatures for IRB approval. Protocol modifications requiring approval may include changes to the title, PI, advisor, funding status or sponsor, subject population composition or size, recruitment, inclusion/exclusion criteria, research site, research procedures and consent/assent process or forms.
2. Submit a request for continuation if the study extends beyond the approval period of one calendar year. This continuation must receive IRB review and approval before the research can continue.
3. Report any adverse events to the IRB Chair promptly. Adverse events are those which are unanticipated and impact the subjects during the course of this research; and
4. Notify the IRB office in writing when your research project is complete.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about th IRB procedures or need any assistance from the Board, please contact Beth McTernan in 219 Cordell North (phone: 405-744-5700, beth.mcternan@okstate.edu).

Sincerely,

Shelia K. Kennison

Shelia Kennison, Chair
Institutional Review Board

VITA

Ban Adnan AL Hakeem

Candidate for the Degree of

Master of Science

Thesis: THE EFFECT OF GRAPE POMACE EXTRACT AS AN ANTIOXIDANT IN GOAT MEAT SAUSAGE

Major Field: Food Science

Biographical:

Education:

Completed the requirements for the Master of Science in Food Science at Oklahoma State University, Stillwater, Oklahoma in December, 2012.

Completed the requirements for the Bachelor of Science in Food Science at Baghdad University, Baghdad, Iraq in 2005.

Experience:

Professional Memberships: